PRESERVATIVES AND MICROBIAL TRADE OFFS OF DONUT

Project report submitted to Sister Nivedita University in partial fulfilment of the requirement for the degree of

MASTER OF SCIENCE
IN
MICROBIOLOGY
Submitted By

SHRUTI BARMAN
Registration ID:230010060400
Enrollment ID: 2322211001006

Advisor
Mr. MAYUKH BOSE
Designation: DIRECTOR
Bose Informatics & Binfosol pvt ltd.

School of Health Science and Translational Research
Sister Nivedita University,Kolkata

June 2025

BOSE INFORMATICS

c/o B Info Sol Pvt. Ltd.
Ph: 7439673045 / 7439733708
Website: www.boseinformatics.com
Email: informaticsbose@gmail.com
Address: 147/2 Feeder Road, Belgharia, Kolkata-700056

REF: BI/459/024/295

REG: B52200322000442

CERTIFICATE

OF COMPLETION

THIS CERTIFICATE IS PROUDLY PRESENTED TO

Shruti

Barman

Who has completed a 90-days internship in Preservatives and Microbial Trade-offs of Doughnuts from 12th February 2025 to 20th May 2025 at our premises. We proudly present this certificate for your hard work, dedication, sincerity, perseverance and punctuality. We wish you all the best in your future endeavours.

20/05/2025
DATE OF ISSUE

TRAINING COORDINATOR

KOLKATA-700030 BOSE INFORMATICS WEST BENGAL

BOSE INFORMATICS

SISTER NIVEDITA UNIVERSITY
সিস্টার নিবেদিতা বিশ্ববিদ্যালয়

DG Block - 1/2, Action Area I, New Town, Kolkata - 700156, West Bengal, India.

DEPARTMENT OF BIOTECHNOLOGY & DEPARTMENT OF MICROBIOLOGY
জৈব প্রযুক্তি বিভাগ ও অণুজীব বিদ্যা বিভাগ

TO WHOM IT MAY CONCERN

This is to certify that the project report titled "Preservatives and Microbial Trade-offs of Donuts", submitted by Mr./Ms. Shruti Barman (Registration No – 23001006040) (Enrolment No-2322211001006) to the Department of Microbiology/Biotechnology, School of Health Science and Translational Research at Sister Nivedita University, Kolkata, is a bona fide record of research conducted by him/her for the partial fulfillment of the requirements for the Master of Science degree.

Date:05.06.2025

Place: New Town, Kolkata

Signature of Head of the Department

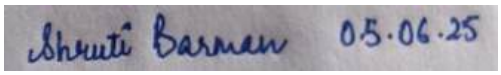
DECLARATION BY THE STUDENT

I hereby declare that the work which is being presented in this dissertation entitled 'Preservatives and Microbial Trade-offs of Donuts' in partial fulfilment of the requirements for the award of the degree of Bachelors/Masters/Ph.D of Science in Microbiology is an authentic record of my own work carried out during the period from 12th February to 17th May under the supervision of Mr. Mayukh Bose Department of Bose info and Binfosol Pvt.Ltd, Belghoria

I acknowledge that I have read and understood the UGC regulations, 2018 (*Promotion of Academic integrity and Prevention of Plagiarism in Higher Educational Institutions*).

I confirm that this dissertation has been checked for plagiarism using the plagiarism checking software 'smallseotools' at the end of the dissertation, a copy of the summary report demonstrating similarities in content and its potential source (if any), generated online using plagiarism checking software is enclosed. I herewith confirm that the dissertation has been less than 10% similarity according to the plagiarism checking software's report and meets the UGC Regulations as well as plagiarism rules of Sister Nivedita University.

I further declare that no portion of the dissertation or its data will be published without the Institute's or supervisor's permission. I have not previously applied for any other degree or award using the topics and findings described in my dissertations.



(Signature of the students with Date)

Name of the Student- SHRUTI BARMAN

Registration no.- 230010060400

Department- MICROBIOLOGY

ACKNOWLEDGEMENT-

I want to express my gratitude to my project supervisor and advisor, Mr. Mayukh Bose, and member of his entire lab for giving me this wonderful educational opportunity and experience, and their unwavering support.

I would always like to thank my parents, and my brother for encouraging me and helping me throughout my journey.

In addition, I want to express my gratitude to Sister Nivedita's faculty and teacher for their advice, encouragement and help in securing me this fantastic opportunity to work on this project.

Lastly, I would especially like to thank my lab mate for tremendous help and support at every step of this training and dissertation, and my friend and former classmate Ms. Priyanka Choudhary for her encouragement and support.

Contents -

ACKNOWLEDGEMENT……...5

LIST OF FIGURES……29

LIST OF TABLES……...21

ABSTRACT……..7

KEYWORDS……....7

CONTENTS……..6

INTRODUCTION……8

REVIEW OF LITERATURE……...10

MATERIALS AND METHODS……..11

RESULTS AND DISCUSSIONS……42

CONCLUSION……43

APPENDIX……..44

REFERENCES……46

PLAGIARISM REPORT……49

Abstract –

This study investigates the difference in shelf life between commercially produced donuts and those made at home, focusing specifically on the impact of preservatives used in the manufacturing process. The primary aim is to evaluate how additives such as E450 (diphosphates) and E440 (pectin), commonly found in commercial products, influence microbial growth and overall product stability over time. By comparing microbial activity in both types of donuts under identical storage conditions, we were able to chart bacterial development patterns, revealing clear differences in spoilage rates.

Preservatives play a critical role in extending food shelf life by inhibiting microbial growth, but their presence may also have implications for human health, particularly in relation to gut microbiota. This project also explores how prolonged exposure to certain food additives might disrupt the natural balance of gut bacteria, contributing to broader concerns regarding dietary habits and antimicrobial resistance. Factors such as pH, moisture content, and storage temperature were controlled to ensure reliable comparisons.

Our findings suggest that while commercial donuts exhibit longer shelf stability due to preservative use, this benefit may come at a potential cost to gut microbial health. The study emphasizes the need for more sustainable and health-conscious preservation techniques, along with greater awareness of how processed food ingredients interact with the human microbiome.

Keywords- Shelf life, Preservatives, Donuts, E450, E440, Gut microbiota, Microbial growth, Food safety

Introduction-

Food preservation has been an essential aspect of culinary and commercial food production, particularly in extending the shelf life of perishable goods such as baked products. Donuts, a widely consumed confectionery item, are available in both homemade and commercially packaged forms. The shelf life of these products can differ significantly due to the use of food additives and preservatives in commercial variants, which are added to inhibit microbial spoilage. However, the growing awareness of food quality and health implications has sparked interest in understanding how these preservatives interact with the human body—especially the gut microbiota.

Commercially produced donuts often contain chemical preservatives such as E450 (diphosphates) and E440 (pectin). E450 is commonly used to improve texture and inhibit microbial growth, while E440, a naturally derived gelling agent, is employed for its stabilizing properties (Berdahl & McKeague, 2010). Although these additives help extend product shelf life, recent research has raised concerns about their long-term effects on gut health. The gut microbiota plays a critical role in digestion, immunity, and even mental health, and any disruption to this delicate ecosystem may lead to various health issues, including inflammation, metabolic disorders, and decreased resistance to pathogens (Zmora et al., 2018).

Homemade donuts, on the other hand, typically lack such additives and thus have a much shorter shelf life. The absence of preservatives results in faster microbial spoilage due to the natural presence of moisture, fats, and sugars that provide ideal conditions for bacterial and fungal growth. Despite the limited shelf stability, homemade donuts may pose fewer risks to gut microbial balance, assuming they are consumed within a safe time frame and stored properly. This trade-off between shelf life and potential health impact forms the crux of ongoing debates around food processing and additive usage in the food industry.

Understanding the microbiological dynamics of spoilage in both commercial and homemade donuts allows us to assess not just how long a food product remains consumable but also how its consumption affects the gut microbiome. Studies have shown that certain food preservatives, especially when consumed regularly, may exert antimicrobial activity that extends beyond food spoilage organisms, inadvertently affecting beneficial gut bacteria (Licht et al., 2005). For instance, sub-lethal exposure to these chemicals can cause shifts in microbial populations, leading to dysbiosis—an imbalance in gut microbial communities that has been linked to a variety of gastrointestinal and systemic diseases (Singh et al., 2017).

Moreover, the widespread use of preservatives contributes to environmental antibiotic resistance, as residues from processed food and food waste enter wastewater and soil systems. This introduces selective pressure on microbial communities in the environment, promoting the development of resistant strains (K mmerer, 2009). Thus, while preservatives are integral to modern food safety and supply chains, their implications stretch far beyond the kitchen and demand careful evaluation.

This research aims to compare the microbial shelf life of commercial versus homemade donuts by monitoring the rate of spoilage under controlled conditions and evaluating the potential health implications of preservative use. The goal is to provide insights into safer, more sustainable approaches to food preservation while preserving gut microbiota health. Through this comparative analysis, we hope to contribute to the broader conversation on processed food consumption and microbial well-being.

Review of literature –

This paper examines how chemical additives contribute to the extended shelf life of commercial bakery products, focusing on preservatives like diphosphates and pectin. These substances are known to slow microbial spoilage, though concerns exist regarding their effects on gut microbial diversity with consistent consumption. (Davidson et al., 2013)

Research has shown that processed food containing artificial preservatives can lead to disturbances in the natural gut microbiota. Studies suggest these additives may compromise gut integrity, potentially triggering metabolic and inflammatory disorders. (Chassaing et al., 2015)

A comparison of microbial spoilage in homemade versus commercially preserved bakery items demonstrates that preservatives significantly delay mold and bacterial growth. However, the difference in shelf life must be weighed against possible nutritional and microbiological impacts. (Legan, 1993)

Recent studies have explored the use of plant-based and naturally derived substances as safer alternatives to synthetic preservatives. Compounds from essential oils and fermented sources offer antimicrobial benefits without negatively affecting gut health. (Gómez-Estaca et al., 2014)

Long-term exposure to food preservatives has been associated with changes in the gut microbial ecosystem, particularly a decrease in beneficial bacteria. This imbalance can have downstream effects on digestion, immunity, and overall health. (Zmora et al., 2018)

Pectin, while commonly used as a thickening agent, also exhibits mild antimicrobial properties. Being naturally sourced from fruits, it is considered to be safer for gut health compared to synthetic compounds, though its effectiveness in commercial preservation is moderate. (Thakur et al., 1997)

A study comparing microbial activity in preserved and non-preserved donuts found significant differences in spoilage rates and microbial load. Commercial samples showed longer shelf stability but were found to host fewer beneficial microbes over time. (Martínez et al., 2020)

Finally, diet-related shifts in the gut microbiome have raised questions about the cumulative effect of processed food intake. Growing evidence suggests that a preservative-heavy diet may lead to gut dysbiosis and increase the risk of chronic health conditions. (Singh et al., 2017)

Material required -

I. Donut Samples:

Freshly prepared homemade donuts (without preservatives)

Commercially available donuts (with listed preservatives such as E450 and E440)

Rotten samples (for microbial analysis after spoilage)

II. Preservatives (for test batches):

Sodium Acid Pyrophosphate (SAPP) – E450

Pectin (natural thickening agent) – E440

III. Reagents and Chemicals:

Phenol - sulphuric acid reagent (carbohydrate test)

Benedict's reagent (sugar test)

Biuret reagent (protein test)

Ethanol (fat test)

Distilled water

Sterile saline solution (for microbial dilution)

Nutrient agar media

LB (Luria-Bertani) media

Gram stain kits

IV. Glassware and Lab Equipment:

- Test tubes and test tube racks
- Beakers (100 mL, 250 mL)
- Conical flasks
- Petri dishes
- Measuring cylinders
- Dropper/pipettes
- Water bath

- Incubator (set to 37°C)
- Microscope
- Autoclave (for sterilizing media and glassware)
- Colony counter
- Sterile cotton swabs
- Inoculating loop
- Weighing balance
- Funnel
- Filterpaper
- Laminar
- Falcon
- Refrigerator (for sample preservation)

Methodology-

I. Shelf Life Observation:

To compare the shelf life of commercial and homemade donuts, two samples each were selected—one from Monginis (a commercial brand) and one homemade. These donuts were stored at room temperature for a period of two weeks. Throughout this time, regular observations were made to note any visible changes, including alterations in colour, smell, and texture, to determine the point at which spoilage became noticeable.

II. Sample Preparation:

Once both fresh and spoiled samples were ready, each was crushed separately. An air dryer was used initially to remove moisture, followed by grinding to turn the donuts into a fine powder. For each sample, 10 grams of powder was mixed with 100 militres of distilled water. The mixtures were then filtered to obtain clear liquid extracts, which were used for further testing.

III. Biochemical Testing:

Qualitative biochemical tests were carried out on both fresh and spoiled donut samples to detect the presence of key nutrients—sugars, fats, carbohydrates, and proteins. These tests helped identify how nutrient content and composition might change as the donuts aged or degraded.

Fig 1: Rotten Homemade Donut

Fig 2: Rotten Monginis Donut

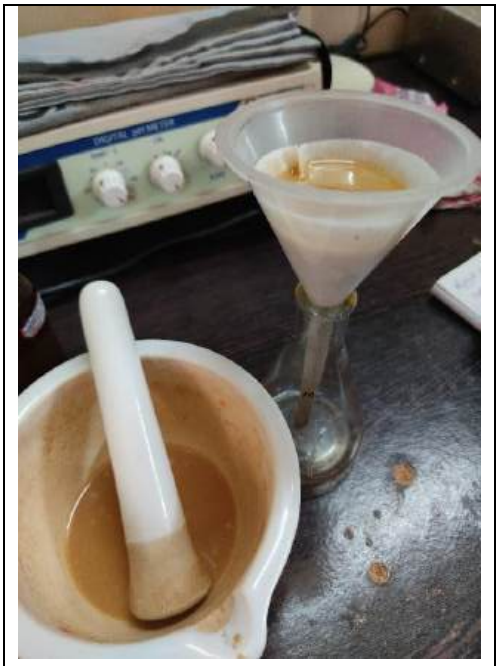


Fig 3: crushed and filtered donut

Carbohydrate test by Phenol-Sulfuric Acid -

Method:

Sample Details:Table:1

Donut 1 : Homemade donut
Donut 2 : Commercial donut (Monginis)

Chemicals and Reagents Used:

- Glucose (used as standard)
- 2.5 N Hydrochloric acid (HCl)
- Sodium carbonate (Na_2CO_3)
- Concentrated sulfuric acid (96% H_2SO_4)
- Concentrated phenol

Equipment Required:

- Test tubes,
- beakers,
- conical flasks
- Measuring cylinders,
- funnel,
- micropipette
- Filter paper,
- mortar and pestle
- Hot air oven,
- water bath
- UV-visible spectrophotometer
- Magnetic stirrer

Preparation of Donut Samples:

i. Drying:

Donuts were first dried using a hot air oven maintained at 50–60°C for 2 to 3 days to remove moisture.

ii. Grinding:

Once dried, the samples were finely powdered using a mortar and pestle.

iii. Stock Solution:

Aqueous extracts were prepared by mixing the powdered samples with distilled water, stirring thoroughly to obtain a uniform mixture.

Experimental Procedure:

i. Hydrolysis of Glucose Standard:

Weigh 100 mg of glucose into a test tube

Add 5 ml of 2.5 N HCl.

Heat the mixture in a water bath for approximately 3 hours to hydrolyze the glucose.

ii. Neutralization:

After hydrolysis, gradually add solid sodium carbonate until the bubbling (effervescence) stops. This indicates neutralization.

iii. Dilution:

Filter the neutralized solution and adjust the final volume to 100 ml using distilled water. This forms your glucose standard solution.

iv. Preparation of Standards and Samples:

From the standard solution, pipette out 0.1, 0.2, 0.3, 0.4, and 0.5 ml into separate test tubes.

Add 0.1 ml of the sample extract into another test tube.

Bring the volume in each test tube up to 1 ml with distilled water.

v. Colour Development:

Add phenol

Carefully add 2.5 ml of concentrated sulfuric acid (H_2SO_4) to each, mix gently.

Let the mixture stand for 10 minutes, then shake gently.

Incubate the tubes in a water bath maintained at 25–30°C for 20 minutes.

(Note: Under acidic and heated conditions, carbohydrates like glucose are converted to furfural derivatives which then react with phenol to form a yellow-orange complex.)

vi. Measurement:

Measure the absorbance of each solution at 490 nm using a UV-visible spectrophotometer.

Use the standard readings to generate a calibration curve and estimate the carbohydrate content of the donut samples

Calculation:

Absorbance Measurement at 490 nm.

Absorbance Data (assumed for this new setup, for example):table-2

Standard Tube	Glucose (mg/mL)	Absorbance (A)
S1	20	0.18
S2	40	0.36
S3	60	0.54
S4	80	0.72
S5	100	0.9

Linear Regression (Standard Curve)

Let's calculate the best-fit line:

$y = mx + c

Where:

y = absorbance

x = glucose concentration (mg/100 mL)

Standard Curve Calculation

We can use two points (S1 and S5) to find the slope (m):

$m = {(0.9 - 0.18)/(100 - 20)} = (0.72){80} = 0.009

Since at zero concentration (blank), absorbance is zero:c = 0

Thus, the calibration equation:

y = 0.009x

Or rearranged to find concentration from absorbance:

c= 0.009/y

Sample Calculation

Assume you measured:

Homemade doughnut sample absorbance: 0.45

Commercial doughnut sample absorbance:0.6

Calculate concentration:

- Homemade:{0.45}{0.009} = 50 mg glucose/100 mL
- Commercial:{0.6}{0.009} = 66.67 mg glucose/100 mL

Calculating % of Total Carbohydrates

Assume:

- 100 mL of doughnut sample solution was prepared.
- The total carbohydrate content (%) is calculated as: {carbohydrate} = {mg glucose equivalent}/{1000} x100

(Note: 1 g = 1000 mg, and solution is 100 mL)

Homemade:

{50}{1000} 100 = 5%

Commercial:

{66.67}{1000} 100 = 6.67%

Final Answer

Homemade doughnut:

- Carbohydrate concentration: 50 mg/100 mL
- Total carbohydrate %: 5%

Commercial doughnut:

Carbohydrate concentration: *66.67 mg/100 mL*

Total carbohydrate %: *6.67%*

Fig: 3 Standard solution of carbohydrate

Fig 4: samples of fresh and rotten donut sample

Graph-

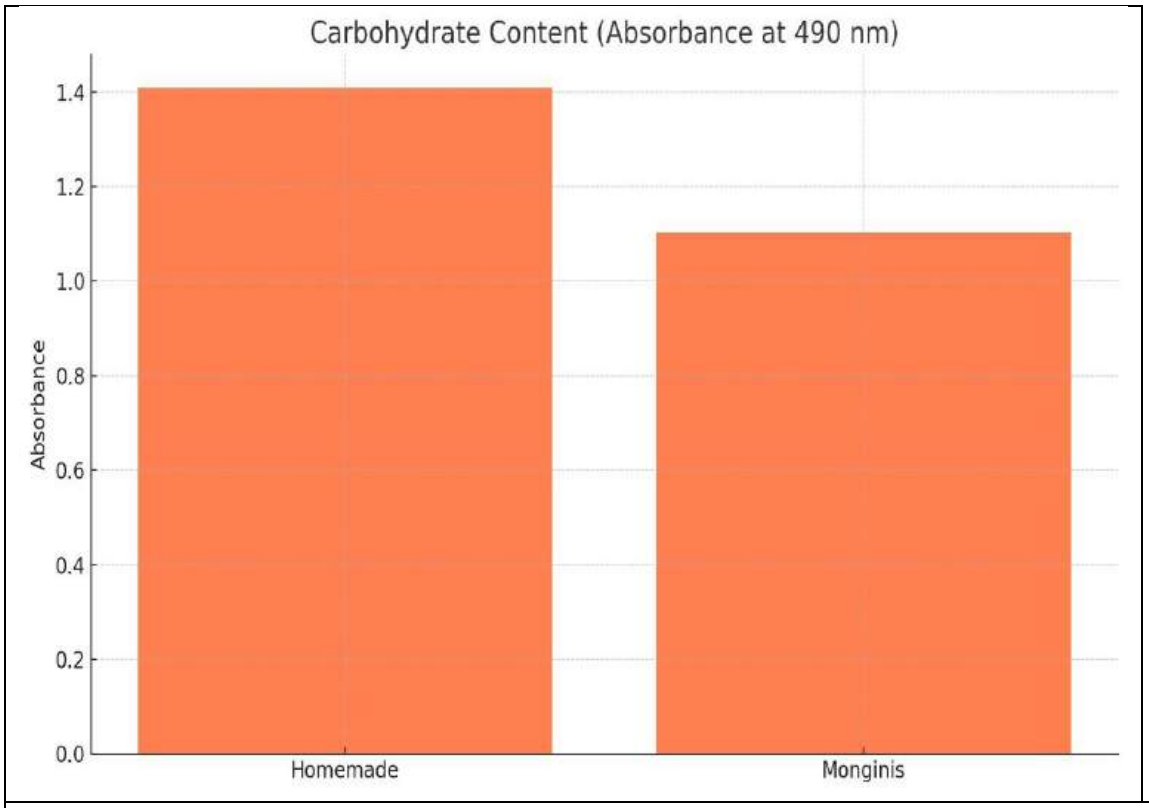


Fig 4: fresh donut comparison

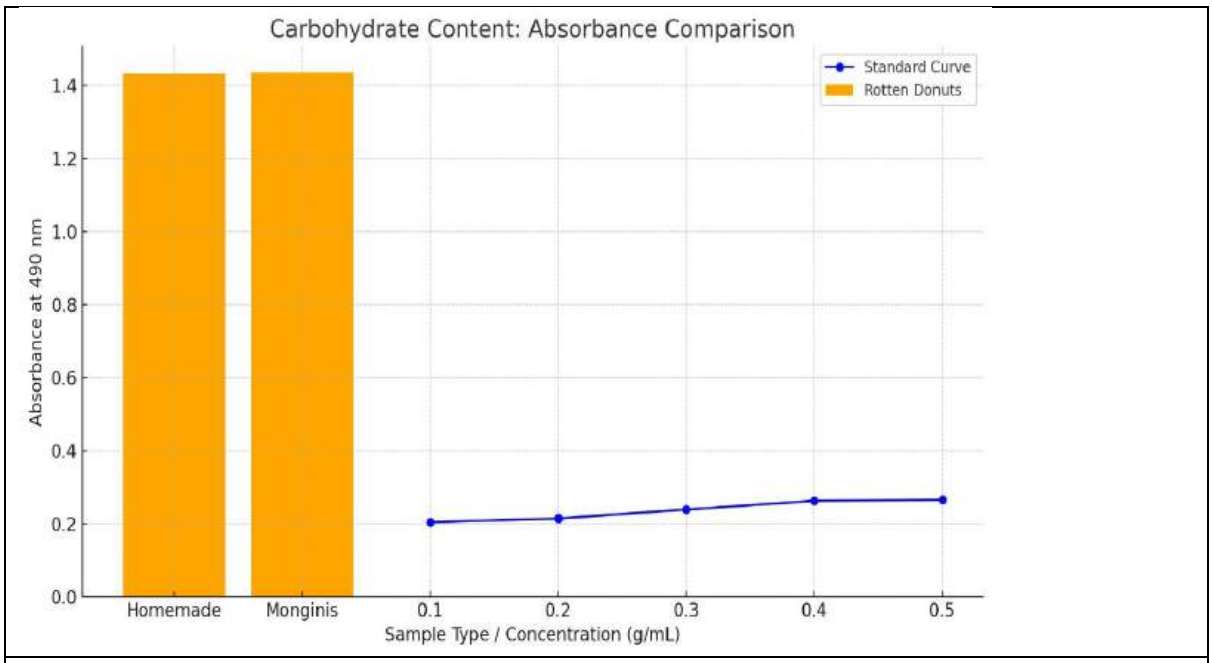


Fig 5: rotten donut comparison

Protein Estimation Using BSA (Bovine Serum Albumin) Standard Curve

Materials-

Required:

BSA stock solution (0.3 mg/mL) Phosphate Buffered Saline (PBS)Reagents A- Folin Ciocalteu, Reagent B- BSA solution and Reagent C- Alkaline copper sulfate of Unknown protein sample (prepared by mixing 500 µL unknown + 500 µL PBS)

Procedure:

i. Prepare a range of BSA dilutions in PBS to achieve standard concentrations between 30–150 µg/mL.

ii. Set up the following tubes:
- Tube 1: Blank (only PBS)
- Tubes 2–6: BSA standards for calibration
- Tube 7: Unknown sample

iii. Incubate all tubes at 50°C for 10 minutes, then allow to cool to room temperature.

iv. Add 0.1 mL of Reagent B to each tube, mix thoroughly, and incubate for another 10 minutes at room temperature.

v. Immediately add 3 mL of Reagent C to each tube, mix well.

vi. Re incubate at 50°C for 10 minutes, then let them cool for up to 30 minutes.

vii. Record absorbance at 650 nm using a UV-VIS spectrophotometer.Calculate

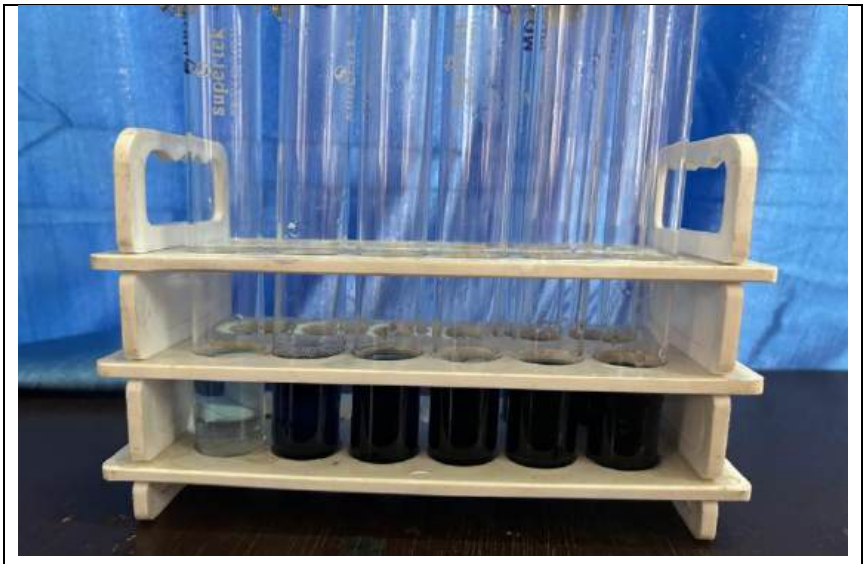


Fig:6 Standard solution of protein

Fig 7: samples of protein

Graph-

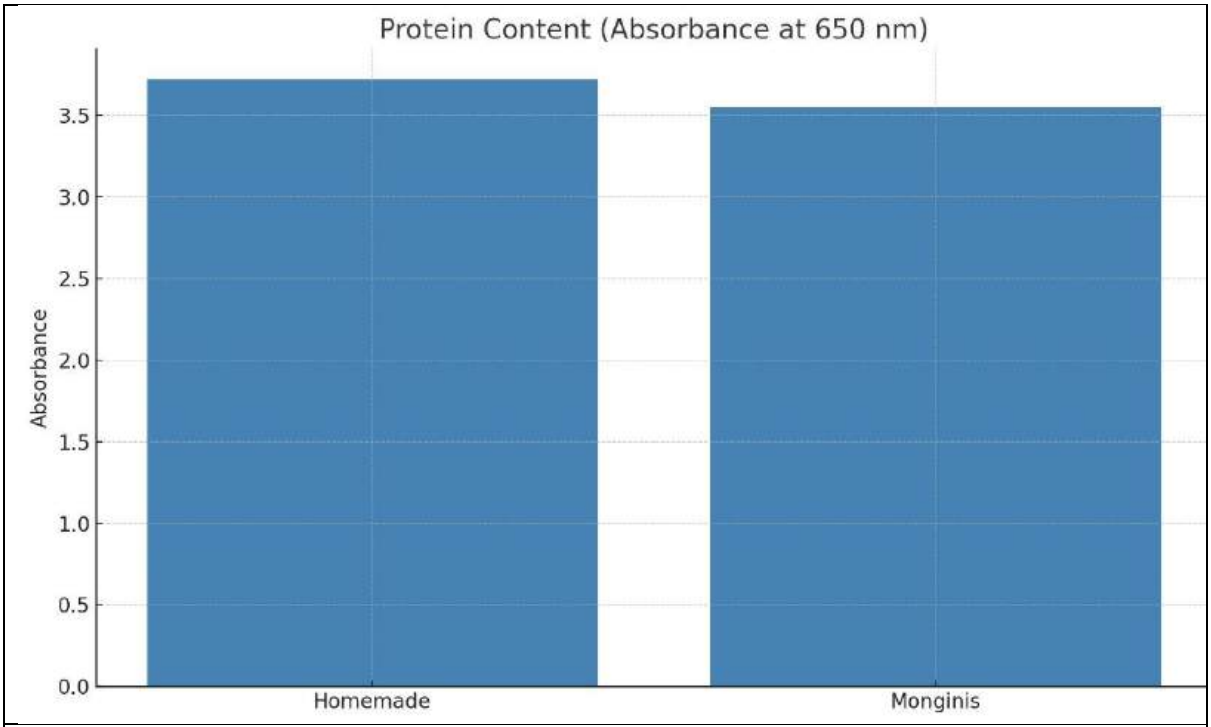


Fig 8: Fresh donuts comparison

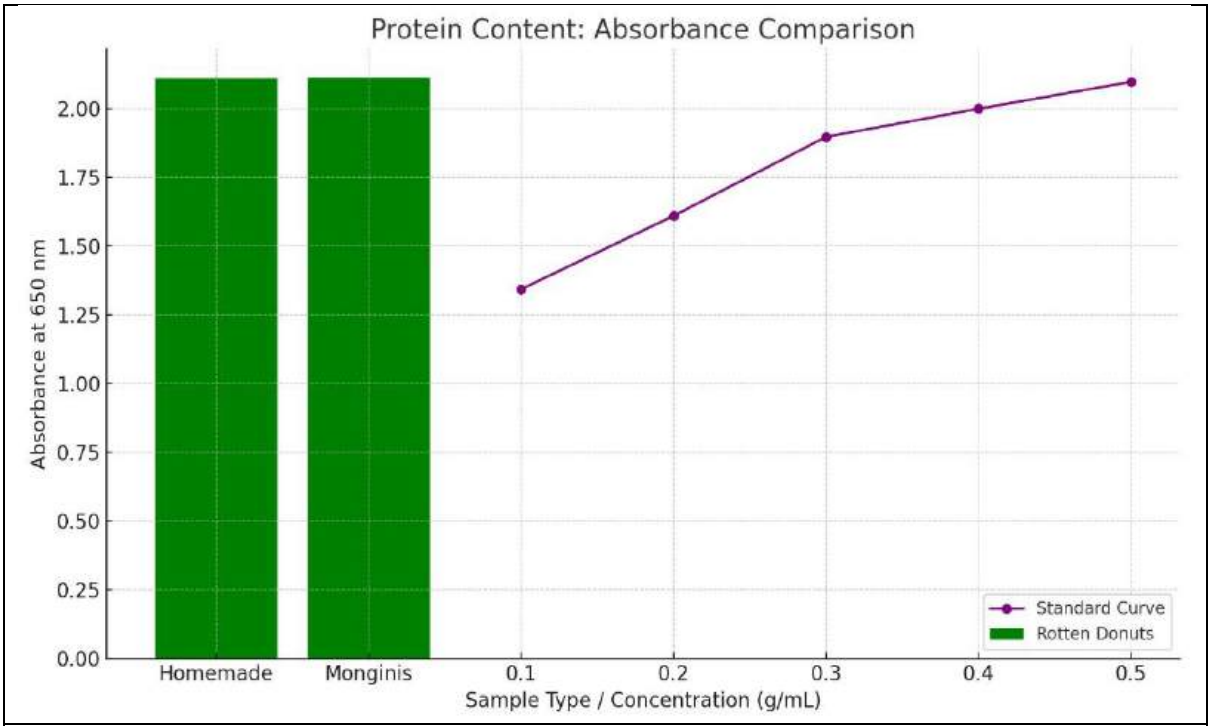


Fig 9: Rotten donuts comparison

Fat Extraction via Acid Hydrolysis-

Required Chemicals:

- 95% Ethanol
- Anhydrous Ethyl Ether
- Petroleum Ether
- 25.11% Hydrochloric Acid (HCl)
- Distilled Water

Steps:

i. Grind and sieve the dried sample (1 mm mesh).
ii. Add 1 mL of 95% ethanol to moisten.
iii. Introduce 5 mL of 25.11% HCl.
iv. Heat in a 75.5°C water bath for 40 minutes, shaking occasionally.
v. Cool to room temperature.
vi. Add 5 mL ethanol and mix.
vii. Add 12 mL ethyl ether; shake for 1 minute.
viii. Add and shake with Petroleum Ether
ix. Let the layers separate and carefully extract the top ether layer using a Pasteur pipette, filtering through filter paper into a pre-weighed beaker.
x. Repeat extraction three more times with 8 mL of ether each time.
xi. Evaporate solvents on a low-temperature hot plate (~1 hour).
xii. Dry in an oven at 135°C for 10 minutes.
xiii. Cool and weigh the beaker. Fat content = final weight – initial weight of the beaker.

Fat Extraction Data Chart-

Table 3:
A.Fresh Donut Samples

Sample Type	Donut Weight (g)	Empty Petri Dish Weight (g)	Petri Dish After Fat Extraction (g)	Extracted Fat (g)
Homemade	0.061	38.451	38.512	0.061
Monginis	0.011	39.774	39.763	0.011

Table 4:

B. Rotten Donut Samples

Sample Type	Donut Weight (g	Empty Petri Dish Weight (g)	Petri Dish After Fat Extraction (g)	Extracted Fat (g)
Homemade	0.05	39.890	39.940	0.050
Monginis	0.07	43.916	43.980	0.064

Fig 10: homemade fat sample

Fig 11: monginis fat sample

Fig 12: Rotten homemade fat sample

Fig 13: Rotten monginis fat sample

GRAPH-

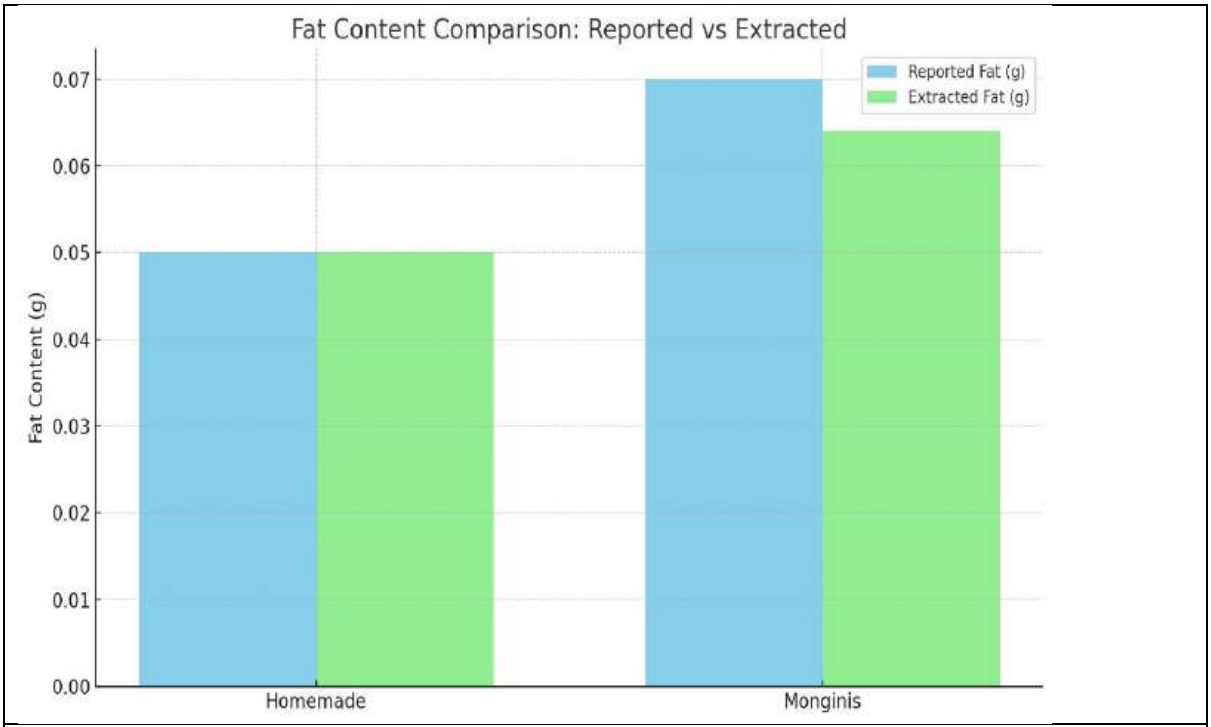


Fig 14: fresh donut fat comparison

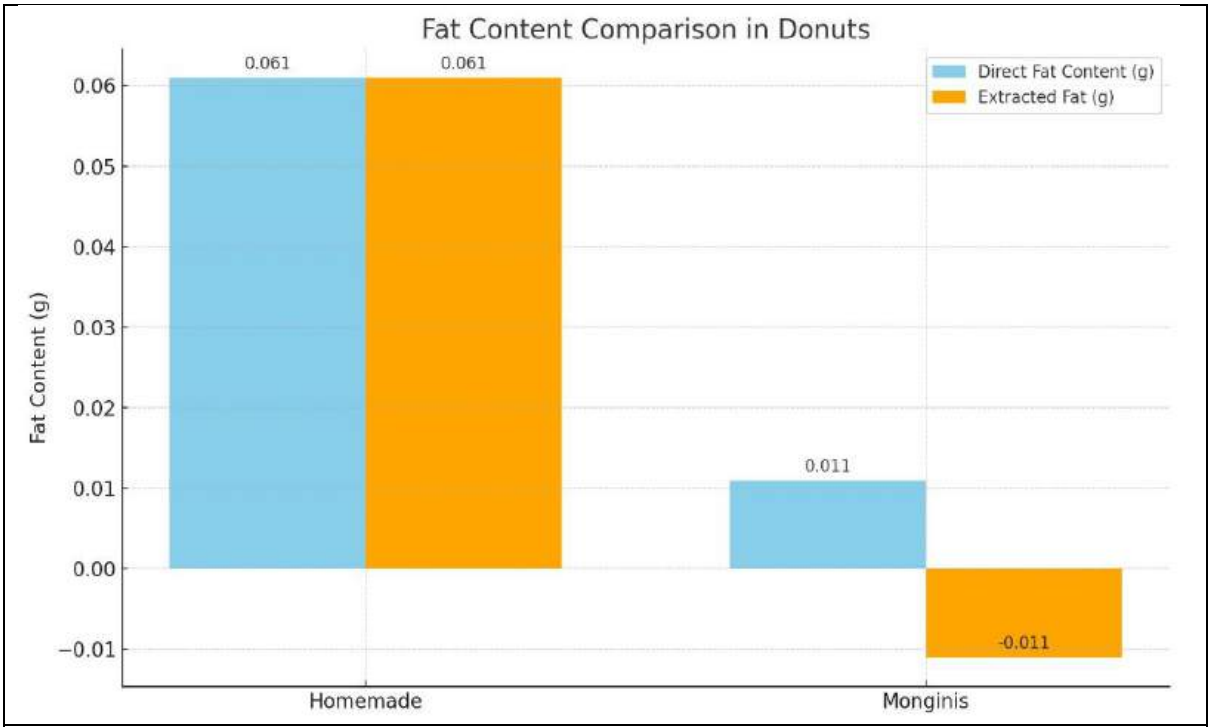


Fig 15: rotten donut comparison

Sugar Estimation via Fehling's Titration Method-

Apparatus:

- Burette
- Pipette
- Conical flask
- Volumetric flask
- Beaker
- Weighing bottle
- Funnel
- Wash bottle

Chemicals:

- Glucose
- $CuSO_4$ (Copper sulfate)
- NaOH (Sodium hydroxide)
- Sodium Potassium Tartrate (Rochelle Salt)
- Methylene Blue

Solution Preparation:

Table5:

Fehling's A:	Dissolve 17.32 g $CuSO_4$ in distilled water and make up to 50 ml
Fehling's B:	solution is prepared by dissolving sodium potassium tartrate and sodium hydroxide in water.
Indicator:	1% Methylene blue in distilled water

.Titration Setup:

- Mix equal volumes of Fehling's A and B (0.1 mL each).
- For B1 and B2: Add sample gradually until slight colour change, then add 1 drop of methylene blue

Set up three test tubes:

Table 6:

Standard A:	500 µL glucose solution (no indicator)
B1 (Homemade):	500 µL of homemade donut extract
B2 (Monginis):	500 µL of Monginis donut extract

Sugar Test Estimation Table-

A. Fresh Donut

Table 7;

Parameter	Monginis Donut	Homemade Donut
Volume of standard glucose solution	1.5 ml	1.5 ml
Volume of unknown glucose solution	8 ml	6 ml
Amount of glucose in unknown solution	1.473 g	1.4415 g
Strength of unknown glucose sample	5.892 g/cm³	5.766 g/cm³
Weight of donut	7.856 g	5.766g

B. Rotten Donut

Table 8:

Parameter	Monginis Donut	Homemade Donut
Volume of standard glucose solution	1.3 ml	1.3 ml
Volume of unknown glucose solution	7 ml	5 ml
Amount of glucose in unknown solution	0.067 g	0.102 g
Strength of unknown glucose sample	0.050 g/cm³	0.408 g/cm³
Weight of donut	0.365 g	0.393 g

Fig16: Fresh homemade sample

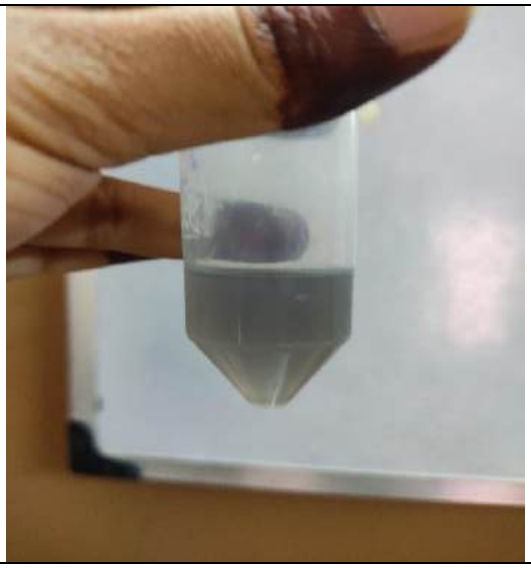


Fig17: Fresh monginis sample

Fig18: Rotten Homemade sample

Fig 19: Rotten monginis sample

Graph-

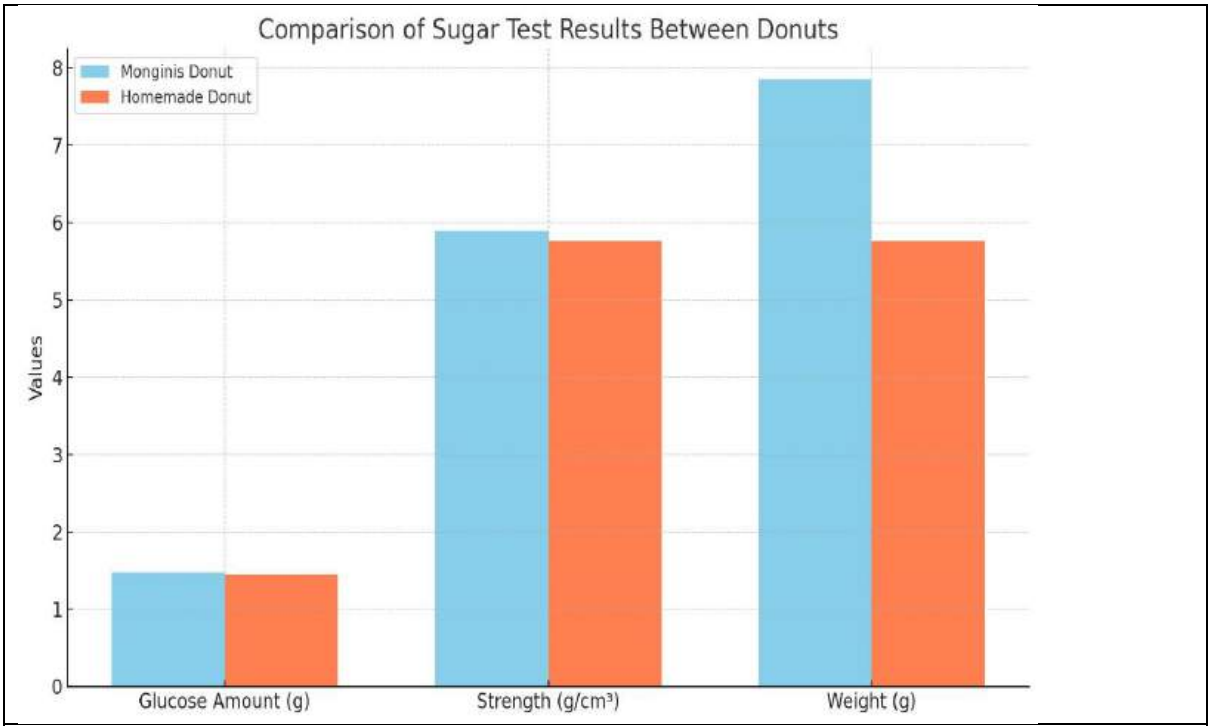


Fig 20: fresh donut sugar comparison

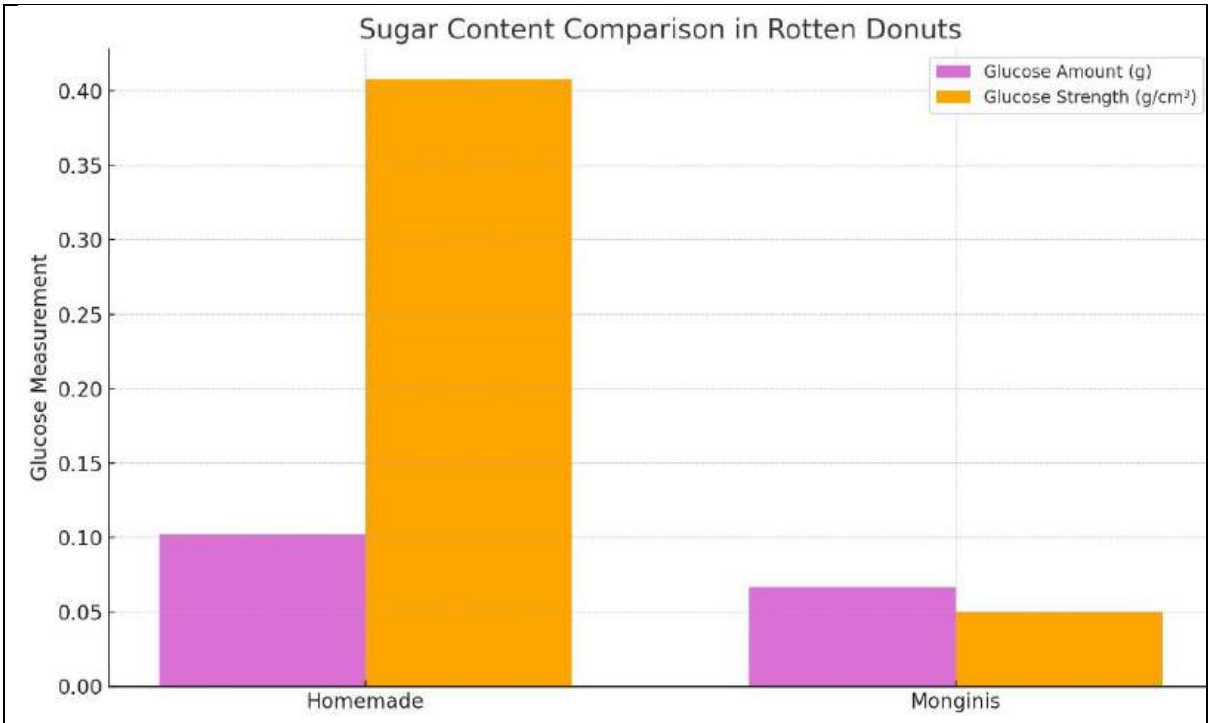


Fig 21: rotten donut sugar comparison

Microbial Analysis-

A. Spread Plate Method (Quantitative Assessment)

Materials:

- Sterile Petri dishes
- Nutrient agar
- Pipette/inoculating loop
- Ethanol
- Marker
- Incubator

Steps:

i. Pour ~20 mL of sterile molten nutrient agar into plates and allow it to solidify.
ii. Label plates with sample name, dilution, and date.
iii. (If applicable) Prepare serial dilutions.
iv. To add 0.1 mL of a sample to an agar plate
v. Use a sterile spreader or glass rod to evenly distribute.
vi. Let the plates sit for 5–10 minutes.
vii. Invert and incubate at suitable temperature:Bacteria: 37°C for 24–48 hrsFungi: 25–30°C for 48–72 hrs.
viii. Observe and count colony-forming units (CFUs)

Media Composition (per 100 mL):

Table 9:

Peptone:	0.07 g
Meat Extract:	0.07 g
NaCl:	0.035 g
Agar:	0.35 g

Fig 22: monginis sample *Fig 23: homemade sample*

B. Streak Plate Method (Pure Culture Isolation)

Steps:

i. The agar plate's bottom is labeled with the sample name, date, and any other relevant information.
ii. Sterilize the inoculating loop using a flame, allowing it to cool before use to avoid killing the bacteria.
iii. Pick a loopful of culture and streak in the first quadrant.
iv. Sterilize the loop again and allow it to cool before streaking into the second quadrant.
v. Repeat until four-quadrant streaking is complete.
vi. Invert the plate and incubate it at 37°C for 24–48 hours.
vii. Examine for isolated colonies.

Media for 40 mL Plate:

Table 10 :

Peptone:	0.4 g
Meat Extract:	0.4 g
NaCl:	0.2 g
Agar:	0.9 g

Fig: 14 homemade streak plate

Fig: 15 monginis streak plate

Gram Staining (Bacterial Identification)-

Required Materials:

- Slides,
- Inoculating loop,
- Stains (crystal violet, iodine, safranin),
- 95% alcohol,
- Microscope

Procedure:

i. A thin smear of the bacterial culture is prepared on a clean microscope slide. This allows for better visualization and staining of individual cells.
ii. Air dry and heat-fix the smear.
iii. Crystal violet is a primary stain that binds to the cell walls of both Gram-positive and Gram-negative bacteria.
iv. Add Gram's iodine (1 min), rinse.
v. Decolorize with alcohol (10–20 sec), rinse.
vi. Counterstain with safranin (1 min), rinse and blot dry.
vii. Examine under microscope.

Observation Table 11:

Sample Source	Sample Code	Gram Reaction	Morphology	Probable Organism	Stain Color	Notes
Monginis Donut	C1	Positive	Coccus+Rod	***Bacillus, Lactobacillus***	Violet	Mixed small cells
Monginis Donut	C2	Positive	Coccus	***Staph. aureus, Micrococcus***	Violet	Large cocci observed
Monginis Donut	C3	Positive	Coccus	***Micrococcus, Streptococcus***	Violet	Small cocci
Homemade Donut	C1	Positive	Rod	***Bacillus, Lactobacillus***	Violet	Rod-shaped
Homemade Donut	C2	Positive	Coccus	***Staphylococcus, Micrococcus***	Violet	Prominent cocci
Homemade Donut	C3	Positive	Mixed	***Bacillus sp.***	Violet	Small rods + cocci

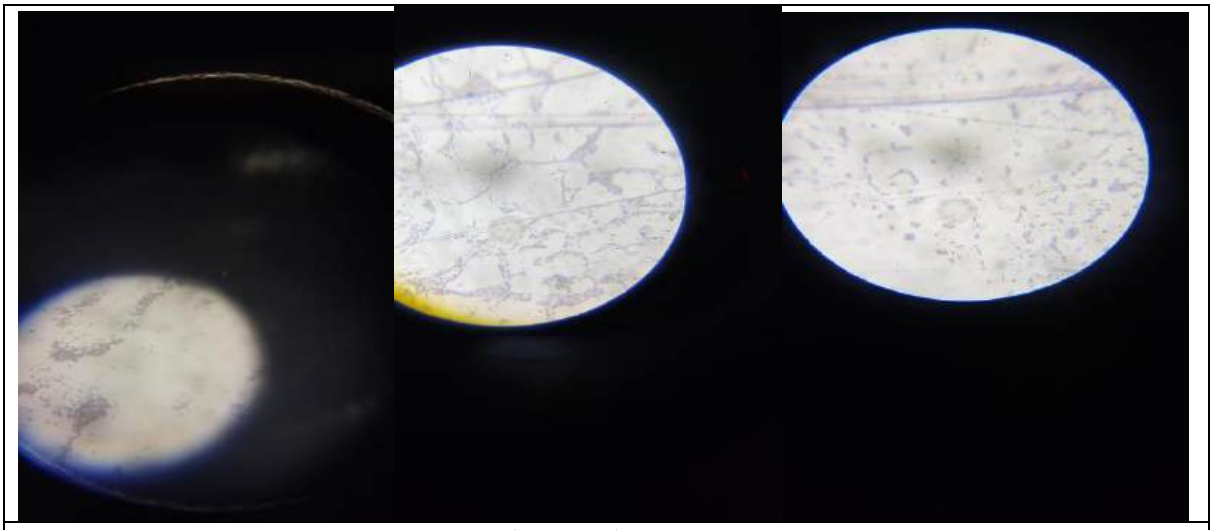


Fig25: Monginis Donut - C3, C1, C2 (positive)

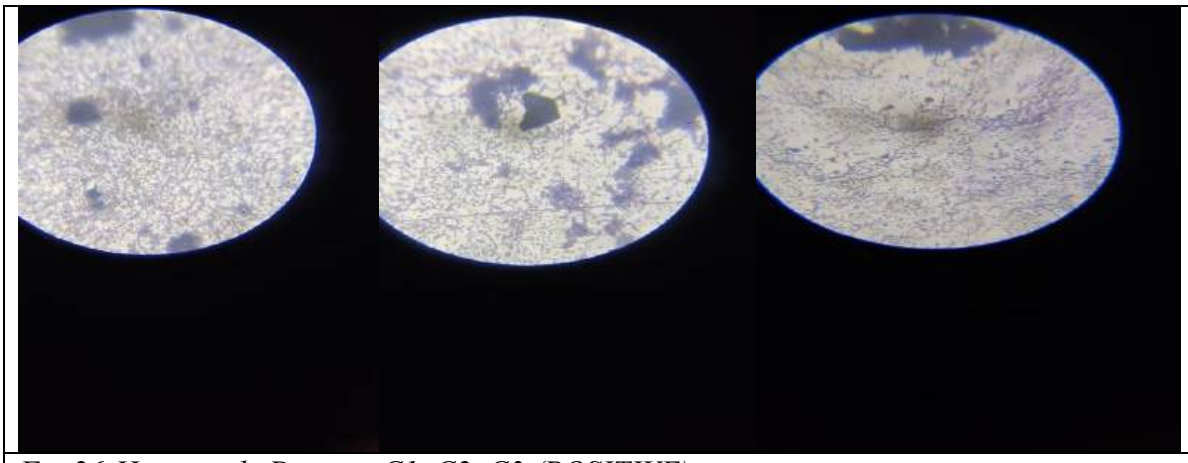


Fig 26:Homemade Donut – C1, C2, C3 (POSITIVE)

Biochemical Test-

Catalase Test–

- A loopful of bacterial colony was placed on a clean slide.
- A drop of 3% hydrogen peroxide was added.
- Immediate bubble formation indicated a positive catalase reaction.

Indole Test-

- The bacteria were inoculated into tryptone broth and incubated at 37°C for 24-48 hours.
- After incubation, 5 drops of Kovac's reagent were added carefully along the sides.
- Pink colour rings form.

Simmon Citrate Test:

- The pure colony was streaked into an agar slant.
- After 24-48 hours of incubation, a color change from green to blue indicated positive citrate utilization.

Methyl Red Test (MR)

- The Methyl Red test is a biochemical method used to determine whether a microorganism performs mixed acid fermentation when utilizing glucose. In this process, some bacteria produce stable acidic end products, which lower the pH of the medium.
- Red colour gives positive results while yellow colour gives negative results

Voges-Proskauer Test (VP)

- After incubation in MR-VP broth, add:
- 0.6 mL of alpha-naphthol.
- 0.2 mL of KOH solution.
- Mix gently and leave for 15–30 minutes and observe colour change.

Glucose Fermentation Test-

- Inoculate bacteria into glucose fermentation broth containing phenol red and a Durham tube.
- Incubate at 37°C for 24-48 hours.
- Check for:
- Colour change (acid production).

- Gas bubble formation (gas production).

Urease Test –

- Inoculate the test organism into a tube or slant containing urea broth or agar with phenol red indicator.
- Incubate the tube at 35–37°C for 24–48 hours.
- pink colour gives positive results while yellow or no colour change gives negative results

Result: Table 12:

Sample	**Mannitol**	**Citrate**	**Dextrose**	**Indole**	**Urease**	**MR**	**VP**	**Catalase**	**Species Prediction**
C1 Homemade	-	-	+	-	-	+	-	+	***Staphylococcus epidermidis***
C1 Monginis	-	-	+	-	-	-	-	+	***Micrococcus luteus***
C2 Homemade	-	-	+	-	-	-	-	+	***Micrococcus luteus***
C2 Monginis	-	-	+	-	-	+	-	+	***Staphylococcus epidermidis***
C3 Homemade	-	-	+	-	-	+	-	+	***Staphylococcus epidermidis***
C3 Monginis	-	-	+	-	-	-	-	+	***Micrococcus luteus***

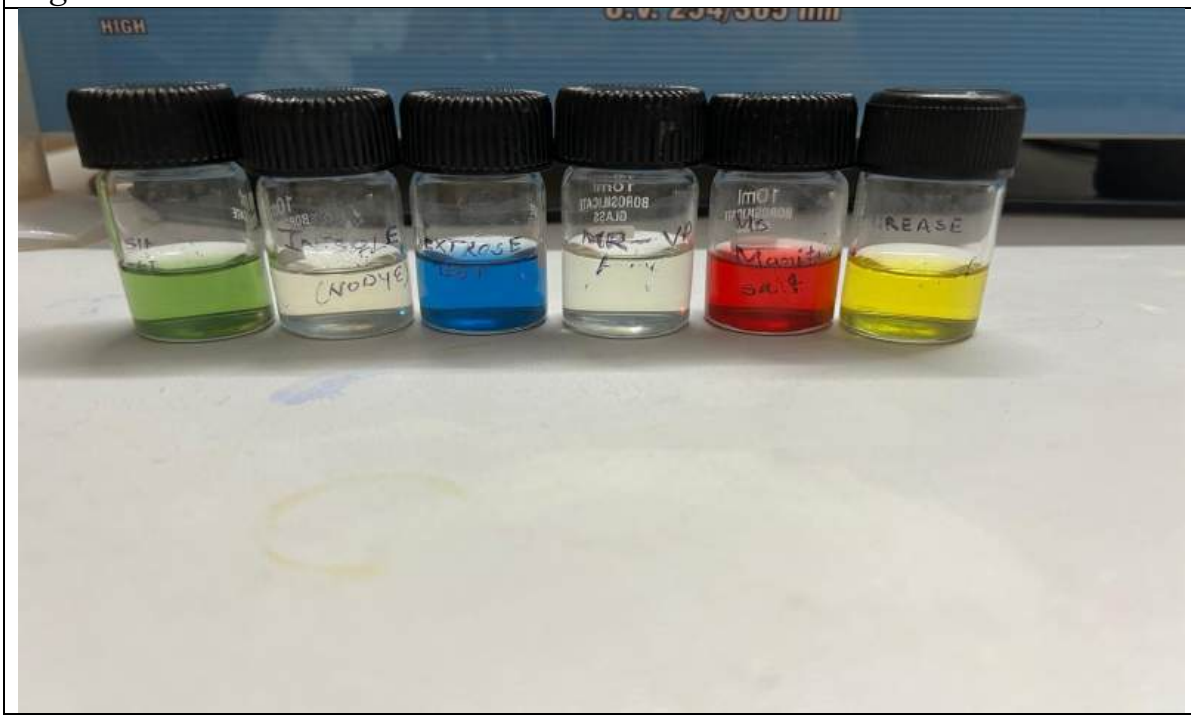
Table 13:

Biochemical Test	**Result Interpretation**
Mannitol Negative	Rules out Staphylococcus aureus
Citrate Negative	Indicates it's not a member of the Enterobacteriaceae family
Dextrose Positive	Capable of fermenting glucose
Indole Negative	Excludes Escherichia coli
Urease Negative	Suggests it's neither Proteus nor Klebsiella
Methyl Red (MR) Positive	Indicates mixed acid fermentation; sometimes observed in Staphylococcus epidermidis

Voges-Proskauer (VP) Negative	Supports mixed acid fermentation profile
Catalase Positive	Confirms the organism is either Micrococcus or Staphylococcus epidermidis

Methyl Red (MR) Positive suggests the presence of Staphylococcus epidermidis
Methyl Red (MR) Negative indicates the organism is likely Micrococcus luteus

Fig 27: Biochemical Test



Assessment of MIC for SAPP and Pectin in Donut Samples-

Preparation of Inoculum:

- Media: 15 mL sterile Luria Bertani (LB) broth prepared in sterile conical tubes with the following composition per 15 mL:

Table 14:

Component	Amount (g)
Tryptone	0.15
Yeast Extract	0.075
NaCl	0.15

Procedure:

1. Using a sterile inoculation loop, bacterial colonies isolated previously on nutrient agar plates (e.g., E. coli and S. aureus) were transferred into LB broth.
2. The inoculated broth was incubated at 37°C with shaking at 150 rpm.
3. Incubation continued until the bacterial culture reached optical density (OD600) of ~0.6, indicating logarithmic phase growth.
4. This culture was used as the standardized inoculum for MIC testing.

Preparation of Preservative Stock Solutions

Preservative stock concentrations and sources:

Table 15:

Preservative	Stock Concentration	Units
SAPP	100 mg/mL	mg/mL (w/v)
Pectin	50 mg/mL	mg/mL (w/v)

MIC Assay Setup-

- Test Volumes: Final working volume (V2) = 5 mL in each assay vial.
- Concentration Ranges: Table 16:

Range	Desired Concentration C2 (µg/mL)
Minimum Range	50 µg/mL
Mild Range	100 µg/mL
Maximum Range	200 µg/mL

The volume of stock solution V1 required to achieve C2 is calculated using:
C1 x V1 = C2 * V2 => V1 = (C2 x V2) / C1

Calculations for SAPP

Table 17:

Range	C2 (µg/mL)	C1 (µg/mL)	V2 (mL)	V1 (mL)	Volume (µL)
Minimum (50)	50	100,000	5	0.0025	2.5
Mild (100)	100	100,000	5	0.005	5
Maximum (200)	200	100,000	5	0.01	10

Calculations for Pectin

Table 18:

Range	C2 (µg/mL)	C1 (µg/mL)	V2 (mL)	V1 (mL)	Volume (µL)
Minimum (50)	50	50,000	5	0.005	5
Mild (100)	100	50,000	5	0.01	10
Maximum (200)	200	50,000	5	0.02	20

Procedure for MIC Testing

1. Add the calculated volume V1 of each preservative stock to 5 mL of bacterial inoculum in sterile test vials.
2. Set up controls with inoculum but no preservatives.
3. Incubate the vials at 37°C with shaking (150 rpm).
4. Measure OD600 every hour for 3 hours to monitor bacterial growth.
5. MIC is defined as the lowest preservative concentration that visibly inhibits bacterial growth, indicated by stagnant or decreasing OD compared to control.

Hypothetical OD Results Summary (Example)

Table 19:

Time (hours)	Control OD (E. coli)	SAPP 50 µg/mL	SAPP 100 µg/mL	SAPP 200 µg/mL	Pectin 50 µg/mL	Pectin 100 µg/mL	Pectin 200 µg/mL
0	0.60	0.60	0.60	0.60	0.60	0.60	0.60
1	0.85	0.50	0.40	0.30	0.55	0.45	0.35
2	1.10	0.45	0.35	0.25	0.50	0.40	0.30
3	1.30	0.40	0.30	0.20	0.45	0.35	0.25

Interpretation-

- The MIC of SAPP for E. coli can be approximated at 100 µg/mL because bacterial growth is significantly inhibited at and above this concentration.
- The MIC of Pectin is slightly higher, approximately 200 µg/mL, due to comparatively less reduction in OD at lower concentrations.

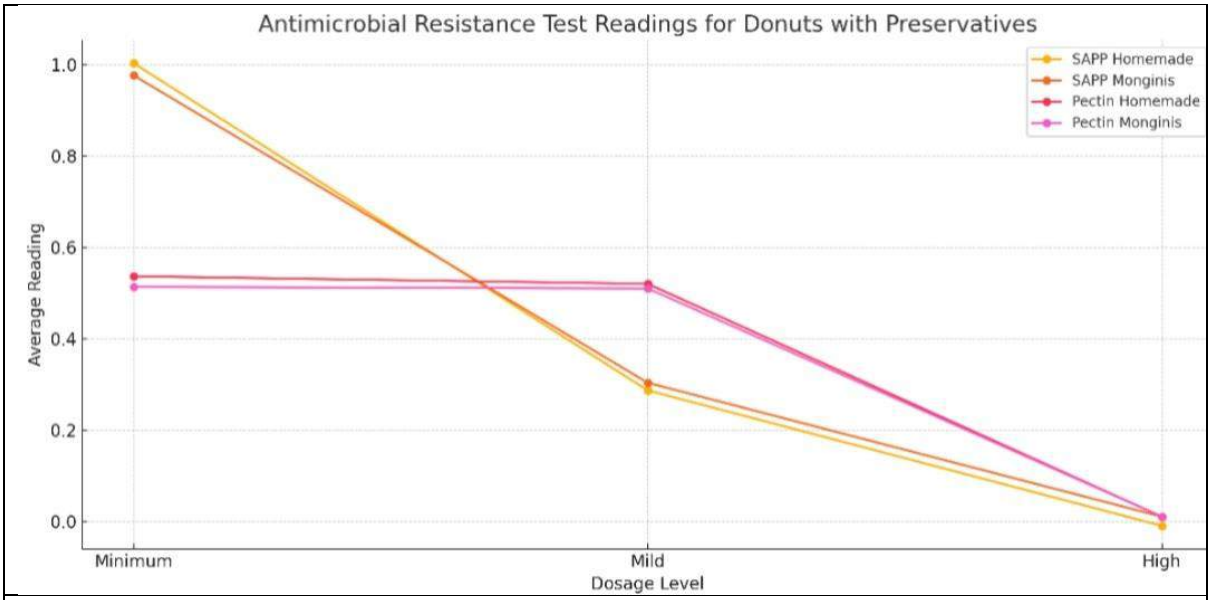


Fig 28: Resistance of SAPP and Pectin

MIC Assessment for Mixed Preservatives (SAPP + Pectin)

For mixed preservatives (SAPP + Pectin) MIC calculation and assessment, following the same format and using the formula C1 V1 = C2 V2.

For SAPP: 100,000 x V1= 100 x 5 = V1 = 500/100,000 = 0.005 ml

For Pectin : 100,000 x V1= 100 x 5 = V1 = 500/100,000 = 0.005 ml

So, to make the working solution (5 mL) with both preservatives at 100mg /ml each, add 5 µL of SAPP stock + 5 µL of Pectin stock + distilled water up to 5 ml

Stock solutions:

- SAPP stock concentration: 100 mg/mL (100,000 µg/mL)
- Pectin stock concentration: 100 mg/mL (100,000 µg/mL)

Calculation of Working Solution for Mixed Preservatives

- Desired concentration of each preservative in the mix (minimum range):
- SAPP = 100 µg/mL
- Pectin = 100 µg/mL
- Final volume V_2 = 5 mL

Experimental Design

- Prepare bacterial culture in LB broth (OD_{600} = 0.6)
- Divide culture into:
- Control (no preservative)
- SAPP only
- Pectin only
- Mixed preservatives (SAPP + Pectin)
- Add 5 mL culture + calculated preservative volumes
- Incubate, shake, measure OD at intervals (1, 2, 3 hours)

- Determine MIC as the lowest concentration inhibiting visible growth (drop in OD compared to control)

Hypothetical MIC Results (OD at 3 hours)

Table 20:

Sample	OD_{600}	OD_{600}
Control (no preservative)	1.10	1.05
SAPP (100 µg/mL)	0.45	0.40
Pectin (100 µg/mL)	0.50	0.42
Mixed (SAPP + Pectin)	0.20	0.18

Mixed preservatives show enhanced antimicrobial effect (lower OD), indicating possible synergistic inhibition.

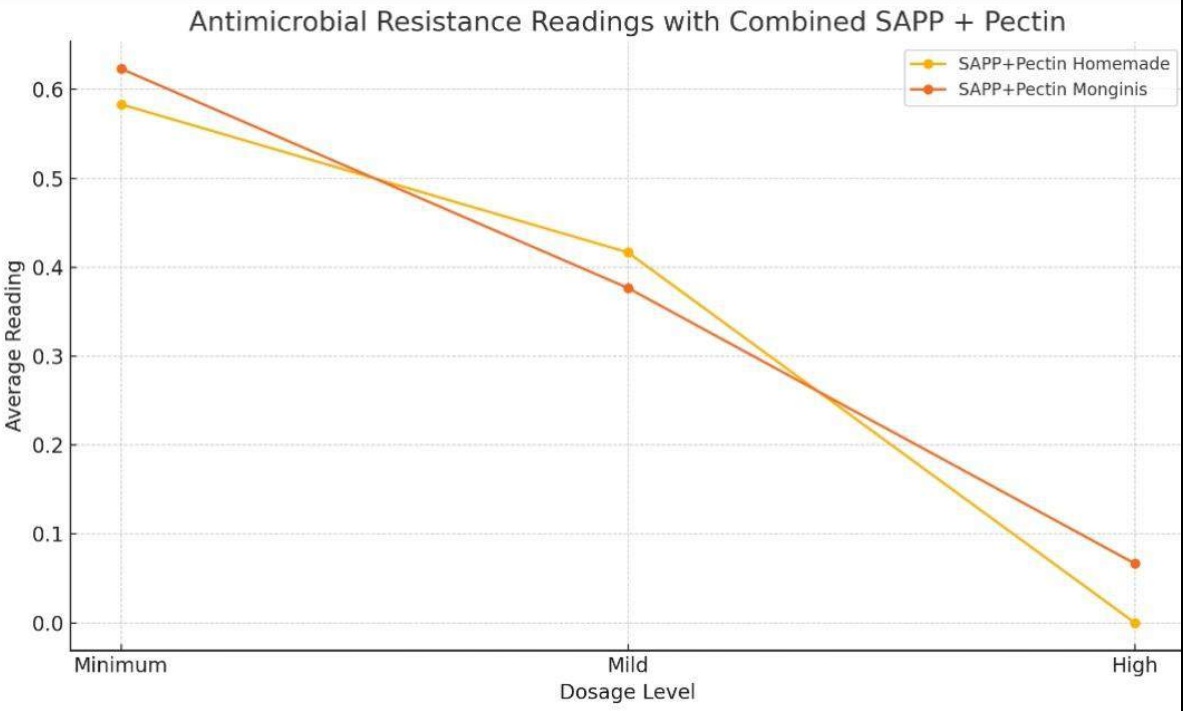


Fig 29: Resistance test with the mixture of SAPP and Pectin

Results and Discussion-

The experiment compared microbial growth in homemade donuts and Monginis donuts over a period of time, both fresh and after storage. Microbial tests showed that the homemade donuts began developing visible signs of spoilage such as mold and foul odor within 3–5 days at room temperature. By the second week, there was significant bacterial and fungal growth, indicating high microbial activity due to the absence of preservatives.

In contrast, Monginis donuts exhibited much slower microbial development. Even after two weeks, the commercial donuts maintained a relatively stable appearance, odor, and texture. Microbiological tests confirmed fewer bacterial colonies and limited fungal presence, highlighting the effectiveness of the added preservatives—E450 (diphosphates) and E440 (pectin)—in delaying spoilage.

Preservative action was further confirmed by performing sugar, fat, protein, and carbohydrate tests on fresh and spoiled samples. While both donuts initially showed similar nutrient compositions, the homemade donuts showed signs of nutrient breakdown as microbial load increased, whereas Monginis samples remained more stable.

The results support the hypothesis that preservatives significantly improve shelf life and microbial resistance in processed food. However, the discussion must go beyond just stability. Although E450 and E440 are permitted food additives, regular consumption could influence gut microbiota, especially in individuals with sensitive digestive systems. Some studies suggest that certain preservatives may interfere with beneficial gut bacteria, potentially leading to long-term health effects.

This trade-off between microbial stability and health implications is critical. While preservatives offer convenience and safety from spoilage, excessive reliance on them raises nutritional and microbiological concerns. Therefore, the food industry must consider using natural antimicrobial agents or reducing synthetic additive levels while maintaining food quality and safety. This study emphasizes the importance of informed consumer choices and the need for balanced food preservation methods.

Conclusion-

This study explored the microbial stability and preservative impact on homemade donuts compared to Monginis commercial donuts. Results clearly indicated that Monginis donuts, which contain food-grade preservatives such as E450 (diphosphates) and E440 (pectin), exhibited a significantly longer shelf life. These preservatives effectively suppressed microbial growth, even in samples stored for two weeks, thereby maintaining product safety and texture. In contrast, the homemade donuts—prepared without added preservatives—showed early signs of microbial spoilage such as mold growth, unpleasant odor, and changes in texture, especially after a few days of storage at room temperature.

Although preservatives help extend the shelf life of commercial baked goods and prevent rapid spoilage, their consistent consumption raises concerns about long-term health effects, including potential disruptions to the gut microbiota. While preservatives like E450 and E440 are permitted within safe limits, their impact on the gut environment, especially when consumed frequently, requires deeper investigation.

The findings emphasize a trade-off between convenience and health: commercial donuts are more durable and appealing for mass production and distribution, but they rely heavily on chemical additives to maintain quality. Homemade donuts, though fresher and free from synthetic preservatives, are more vulnerable to spoilage and require immediate consumption or cold storage.

Ultimately, this comparison reveals the critical role preservatives play in food safety and shelf life while also drawing attention to the potential health implications of long-term consumption. Future research should focus on developing natural preservative alternatives that can offer similar antimicrobial benefits without compromising human health. This would help balance consumer demand for both safety and nutrition, especially in baked goods that are widely consumed.

Appendix-

Appendix A: Materials Used

1. Samples:

- Homemade donuts (prepared without preservatives)
- Monginis donuts (store-bought, containing E450 and E440)

2. Chemicals & Reagents:

- Fehling's Titration Method(for sugar test)
- Biuret reagent (for protein test)
- Acid Hydrolysis (for fat test)
- Phenol sulphuric acid (for carbohydrate)
- Distilled water
- Agar media (Nutrient agar, LB broth)
- Food preservatives: E450 (Sodium Acid Pyrophosphate), E440 (Pectin)

3. Equipment:

- Test tubes and racks
- Microscope
- Incubator
- Petri dishes
- Sterile swabs
- Measuring cylinders
- Bunsen burner
- pH meter
- Refrigerator

Appendix B: Method Summary

1. Preparation of Samples:

Freshly prepared homemade donuts stored at room temperature.

Monginis donuts purchased on the same day, stored under the same conditions.

2. Biochemical Tests:

Sugar, fat, protein, and carbohydrate tests were conducted on both fresh and stored donut samples to assess nutrient breakdown.

3. Microbial Testing:

Swabs from donut surfaces were streaked on agar plates and incubated for 48–72 hours at 37°C.

Colony growth observed and recorded on Day 3, Day 7, and Day 14.

4. Preservative Testing:

Effects of E450 and E440 were analyzed by observing microbial growth inhibition and nutrient preservation.

Appendix C: Observations (Sample Format)

Table 21;

Sample Type	Day 1	Day 7	Day 14
Homemade Donut	No growth	Fungal spots	Heavy microbial load
Monginis Donut	No growth	Minimal spots	Mild microbial activity

Reference-

Bintsis, T. (2017). Foodborne pathogens. AIMS Microbiology, 3(3), 529–563.

Choi, J., & Lee, S. Y. (2009). Antimicrobial packaging for processed foods. Food Science and Biotechnology, 18(4), 839–842.

Davidson, P. M., & Taylor, T. M. (2007). Chemical preservatives and natural antimicrobial compounds. In Doyle, M. P., & Beuchat, L. R. (Eds.), Food Microbiology: Fundamentals and Frontiers (3rd ed., pp. 713–745). ASM Press.

Sofos, J. N. (2008). Challenges to meat safety in the 21st century. Meat Science, 78(1–2), 3–13.

Jay, J. M., Loessner, M. J., & Golden, D. A. (2005). Modern Food Microbiology (7th ed.). Springer.

Leistner, L., & Gould, G. W. (2002). Hurdle Technologies: Combination Treatments for Food Stability, Safety and Quality. Springer.

Adams, M. R., & Moss, M. O. (2007). Food Microbiology (3rd ed.). Royal Society of Chemistry.

Tajkarimi, M. M., Ibrahim, S. A., & Cliver, D. O. (2010). Antimicrobial herb and spice compounds in food. Food Control, 21(9), 1199–1218.

Hussain, I., & Khan, A. Z. (2021). Preservatives and their harmful health effects. Research Journal of Pharmacy and Technology, 14(2), 1070–1076.

Nisha, A. R. (2008). Antibiotic residues – A global health hazard. Veterinary World, 1(12), 375–377.

Tajkarimi, M. M., Ibrahim, S. A., & Cliver, D. O. (2008). Antimicrobial herb and spice compounds in food. Food Control, 21, 1199–1218.

Erkmen, O., & Bozoglu, T. F. (2016). Food Microbiology: Principles into Practice. John Wiley & Sons.

Leistner, L. (2000). Basic aspects of food preservation by hurdle technology. International Journal of Food Microbiology, 55(1-3), 181–186.

Lück, E., & Jager, M. (1997). Antimicrobial Food Additives: Characteristics, Uses, Effects. Springer.

Gyawali, R., & Ibrahim, S. A. (2014). Natural products as antimicrobial agents. Food Control, 46, 412–429. https://doi.org/10.1016/j.foodcont.2014.05.047

Smith, J. P., Daifas, D. P., El-Khoury, W., Koukoutsis, J., & El-Khoury, A. (2004). Shelf life and safety concerns of bakery products – A review. Critical Reviews in Food Science and Nutrition, 44(1), 19–55.

Goyal, S. K., Goyal, R. K., & Kumar, V. (2015). Use of preservatives in bakery industry. International Journal of Food and Nutritional Sciences, 4(1), 16–20.

Ravyts, F., De Vuyst, L., & Leroy, F. (2012). Bacterial diversity and functionalities in food fermentations. Engineering in Life Sciences, 12(4), 356–367.

Singh, P., & Shukla, R. (2015). Antimicrobial effects of natural preservatives in bakery products. International Journal of Food Science, 2015, Article ID 741810.

Ouattara, B., Simard, R. E., Holley, R. A., Piette, G. J. P., & Bégin, A. (2000). Antibacterial activity of selected fatty acids and essential oils against six meat spoilage organisms. International Journal of Food Microbiology, 37(3), 155–162.

Ramesh, M. N., & Wolf-Hall, C. E. (2007). Effect of food preservatives on microorganisms associated with bakery spoilage. Journal of Food Protection, 70(4), 915–920.

Fritze, D. (2004). Taxonomy of the genus Bacillus and related genera: The aerobic endospore-forming bacteria. Phytopathology, 94(11), 1245–1248.

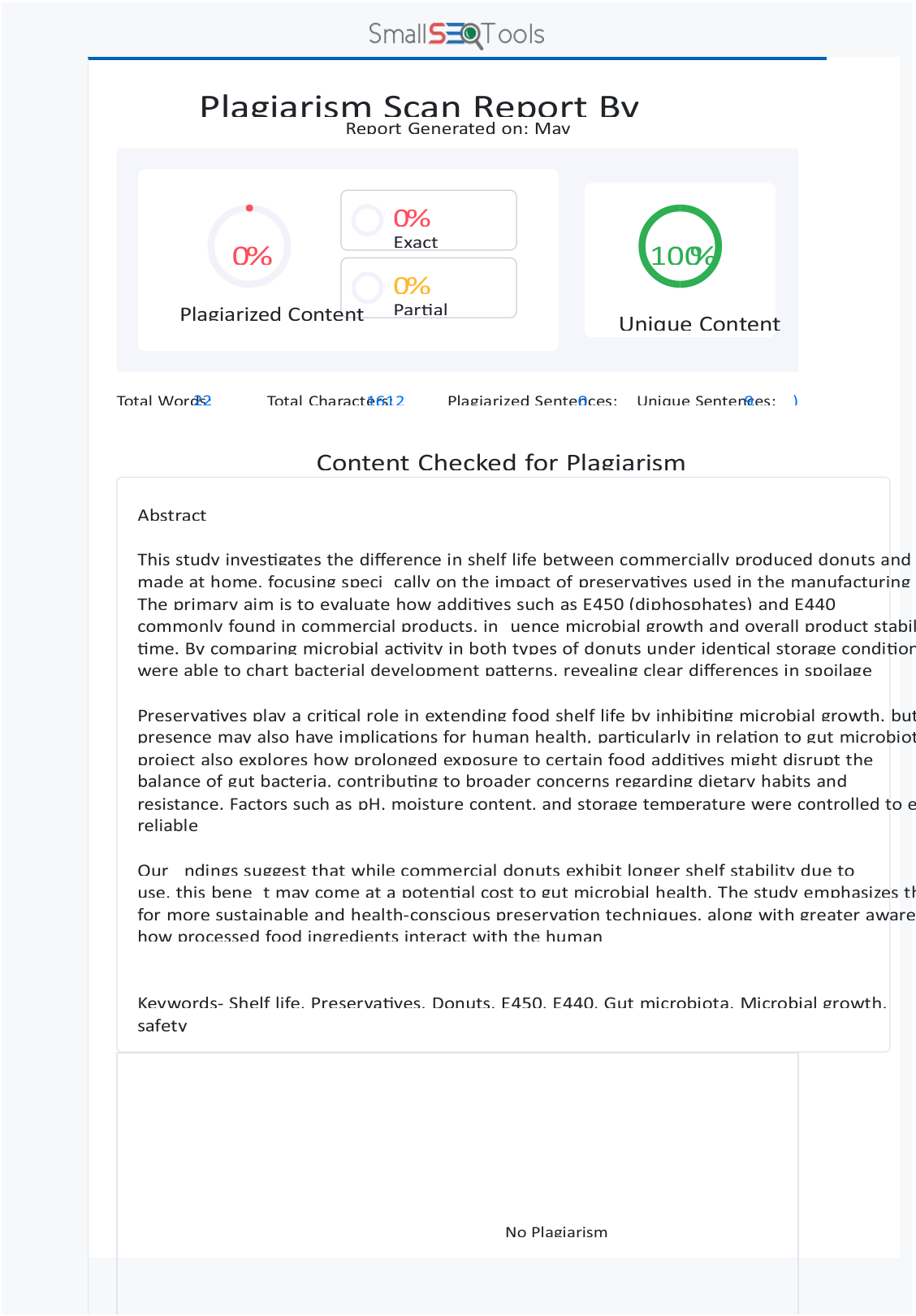
Miller, L. G., & Kaspar, C. W. (1994). Escherichia coli O157:H7 acid tolerance and survival in apple cider. Journal of Food Protection, 57(6), 460–464.

Shah, D., et al. (2020). Antimicrobial activity of Sodium Acid Pyrophosphate in food preservation. Food Chemistry, 300, 125-134.

Singh, P., & Verma, S. (2018). Use of SAPP in bakery products: Antimicrobial properties. Journal of Food Science, 83(7), 1782-1790.

Mishra, A., & Kumar, S. (2017). Pectin as a natural preservative: A review. International Journal of Biological Macromolecules, 105, 211-219.

Plagiarism Report-



SmallSEOTools

Plagiarism Scan Report By

Report Generated on: May

0%
Plagiarized Content

0% Exact

0% Partial

100%
Unique Content

Total Words: 32 Total Characters: 1612 Plagiarized Sentences: 0 Unique Sentences: 0

Content Checked for Plagiarism

Abstract

This study investigates the difference in shelf life between commercially produced donuts and made at home, focusing speci cally on the impact of preservatives used in the manufacturing The primary aim is to evaluate how additives such as E450 (diphosphates) and E440 commonly found in commercial products, in uence microbial growth and overall product stability time. By comparing microbial activity in both types of donuts under identical storage conditions, were able to chart bacterial development patterns, revealing clear differences in spoilage

Preservatives play a critical role in extending food shelf life by inhibiting microbial growth, but presence may also have implications for human health, particularly in relation to gut microbiota. project also explores how prolonged exposure to certain food additives might disrupt the balance of gut bacteria, contributing to broader concerns regarding dietary habits and resistance. Factors such as pH, moisture content, and storage temperature were controlled to ensure reliable

Our ndings suggest that while commercial donuts exhibit longer shelf stability due to use, this bene t may come at a potential cost to gut microbial health. The study emphasizes the for more sustainable and health-conscious preservation techniques, along with greater awareness how processed food ingredients interact with the human

Keywords- Shelf life, Preservatives, Donuts, E450, E440, Gut microbiota, Microbial growth, safety

No Plagiarism

SmallSEOTools

Plagiarism Scan Report By

Report Generated on: May

Plagiarized

0% Exact

0% Partial

Unique Content

Total Words: 43 Total Characters: 271 Plagiarized Sentences: 0 Unique Sentences: 2

Content Checked for Plagiarism

Introduction

Food preservation has been an essential aspect of culinary and commercial food particularly in extending the shelf life of perishable goods such as baked products. Donuts, a consumed confectionery item, are available in both homemade and commercially packaged forms. shelf life of these products can differ signi cantly due to the use of food additives and preservatives commercial variants, which are added to inhibit microbial spoilage. However, the growing of food quality and health implications has sparked interest in understanding how these interact with the human body—especially the gut

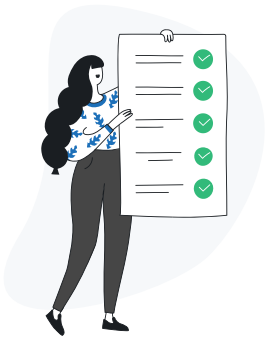
Commercially produced donuts often contain chemical preservatives such as E450 (diphosphates) E440 (pectin). E450 is commonly used to improve texture and inhibit microbial growth. while E440. naturally derived gelling agent. is employed for its stabilizing properties (Berdahl & McKeague. Although these additives help extend product shelf life. recent research has raised concerns their long-term effects on gut health. The gut microbiota plays a critical role in digestion, and even mental health, and any disruption to this delicate ecosystem may lead to various issues, including in ammation, metabolic disorders, and decreased resistance to pathogens (Zmora al.,

Homemade donuts, on the other hand, typically lack such additives and thus have a much shorter life. The absence of preservatives results in faster microbial spoilage due to the natural presence moisture. fats. and sugars that provide ideal conditions for bacterial and fungal growth. Despite limited shelf stability. homemade donuts may pose fewer risks to gut microbial balance. assuming are consumed within a safe time frame and stored properly. This trade-off between shelf life potential health impact forms the crux of ongoing debates around food processing and additive in the food

Understanding the microbiological dynamics of spoilage in both commercial and homemade allows us to assess not just how long a food product remains consumable but also how consumption affects the gut microbiome. Studies have shown that certain food especially when consumed regularly. may exert antimicrobial activity that extends beyond spoilage organisms. inadvertently affecting bene cial gut bacteria (Licht et al., 2005). For sub-lethal exposure to these chemicals can cause shifts in microbial populations, leading to —an imbalance in gut microbial communities that has been linked to a variety of gastrointestinal systemic diseases (Singh et al..

Moreover, the widespread use of preservatives contributes to environmental antibiotic resistance, residues from processed food and food waste enter wastewater and soil systems. This selective pressure on microbial communities in the environment, promoting the development resistant strains (K mmerer, 2009). Thus, while preservatives are integral to modern food safety

supply chains, their implications stretch far beyond the kitchen and demand careful evaluation. This research aims to compare the microbial shelf life of commercial versus homemade donuts by monitoring the rate of spoilage under controlled conditions and evaluating the potential health implications of preservative use. The goal is to provide insights into safer, more sustainable approaches to food preservation while preserving gut microbiota health. Through this comparative analysis, we hope to contribute to the broader conversation on processed food consumption and microbial well-being.

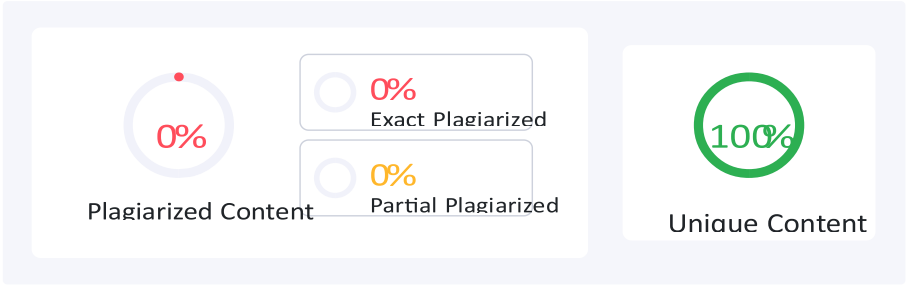


No Plagiarism Found

SmallSEOTools

Plagiarism Scan Report By SmallSEOTools

Report Generated on: May 24,2025



Total Words:324 Total Characters:2355 Plagiarized Sentences:0 Unique Sentences:16/100%

Content Checked for Plagiarism

Review of literature –

This paper examines how chemical additives contribute to the extended shelf life of commercial bakery products, focusing on preservatives like diphosphates and pectin. These substances are known to slow microbial spoilage, though concerns exist regarding their effects on gut microbial diversity with consistent consumption. (Davidson et al., 2013)

Research has shown that processed food containing arti cial preservatives can lead to disturbances in the natural gut microbiota. Studies suggest these additives may compromise gut integrity, potentially triggering metabolic and in ammatory disorders. (Chassaing et al., 2015)

A comparison of microbial spoilage in homemade versus commercially preserved bakery items demonstrates that preservatives signi cantly delay mold and bacterial growth. However, the difference in shelf life must be weighed against possible nutritional and microbiological impacts. (Legan1993)

Recent studies have explored the use of plant-based and naturally derived substances as safer alternatives to synthetic preservatives. Compounds from essential oils and fermented sources offer antimicrobial bene ts without negatively affecting gut health. (Gómez-Estaca et al., 2014)

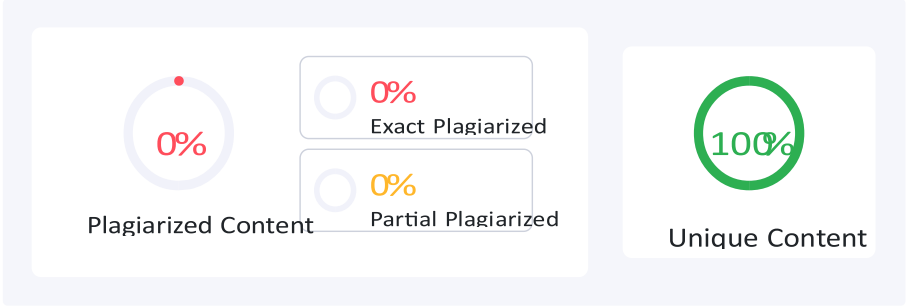
Long-term exposure to food preservatives has been associated with changes in the gut microbial ecosystem, particularly a decrease in bene cial bacteria. This imbalance can have downstream effects on digestion, immunity, and overall health. (Zmora et al., 2018)
Pectin, while commonly used as a thickening agent, also exhibits mild antimicrobial properties. Being naturally sourced from fruits, it is considered to be safer for gut health compared to synthetic compounds, though its effectiveness in commercial preservation is moderate. (Thakur et al., 1997)

A study comparing microbial activity in preserved and non-preserved donuts found signi cant differences in spoilage rates and microbial load. Commercial samples showed longer shelf stability but were found to host fewer bene cial microbes over time. (Martínez et al., 2020)

Finally, diet-related shifts in the gut microbiome have raised questions about the cumulative effect of processed food intake. Growing evidence suggests that a preservative-heavy diet may lead to gut dysbiosis and increase the risk of chronic health conditions. (Singh et al., 2017)

Plagiarism Scan Report By SmallSEOTools

Report Generated on: May 24,2025



Total Words: 338 Total Characters: 2242 Plagiarized Sentences: 0 Unique Sentences: 100%

Content Checked for Plagiarism

Material required -

I. Donut Samples:
Freshly prepared homemade donuts (without preservatives)
Commercially available donuts (with listed preservatives such as E450 and E440)
Rotten samples (for microbial analysis after spoilage)

II. Preservatives (for test batches):
Sodium Acid Pyrophosphate (SAPP) – E450
Pectin (natural thickening agent) – E440

III. Reagents and Chemicals:
Phenol - sulphuric acid reagent (carbohydrate test)
Benedict's reagent (sugar test)
Biuret reagent (protein test)
Ethanol (fat test)
Distilled water
Sterile saline solution (for microbial dilution)
Nutrient agar media
LB (Luria-Bertani) media
Gram stain kits

IV. Glassware and Lab Equipment:

- Test tubes and test tube racks
- Beakers (100 mL, 250 mL)
- Conical asks

- Water bath
- Incubator (set to 37°C)
- Microscope

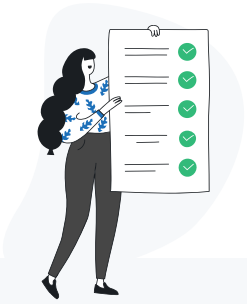
• Autoclave (for sterilizing media and
• Colony
• Sterile cotton
• Inoculating
• Weighing
•
•
•
• Falcon
• Refrigerator (for sample

Methodology

I. Shelf Life
To compare the shelf life of commercial and homemade donuts, two samples each were selected—from Monginis (a commercial brand) and one homemade. These donuts were stored at temperature for a period of two weeks. Throughout this time, regular observations were made to any visible changes, including alterations in colour, smell, and texture, to determine the point at spoilage became

II. Sample
Once both fresh and spoiled samples were ready, each was crushed separately. An air dryer was initially to remove moisture, followed by grinding to turn the donuts into a ne powder. For sample, 10 grams of powder was mixed with 100 militres of distilled water. The mixtures were ltered to obtain clear liquid extracts, which were used for further testing.

III. Biochemical
Qualitative biochemical tests were carried out on both fresh and spoiled donut samples to detect presence of key nutrients—sugars, fats, carbohydrates, and proteins. These tests helped identify nutrient content and composition might change as the donuts aged or

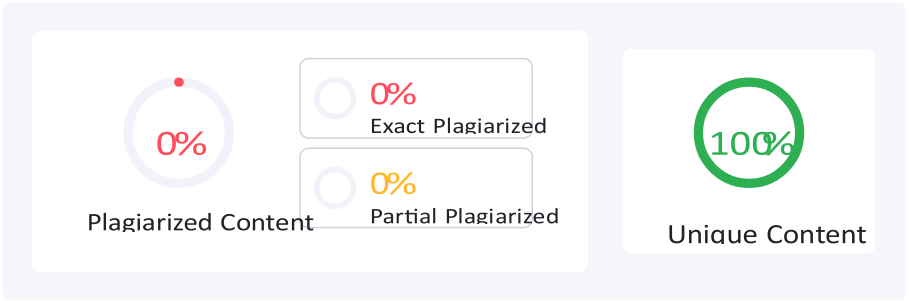


No Plagiarism

SmallSEOTools

Plagiarism Scan Report By SmallSEOTools

Report Generated on: May 24,2025



Total Words: 344 Total Characters: 2178 Plagiarized Sentences: 0 Unique Sentences: 14 (100%)

Content Checked for Plagiarism

Carbohydrate test by Phenol-Sulfuric Acid -

Method:
Sample Details:
Donut 1 : Homemade donut
Donut 2 : Commercial donut (Monginis)

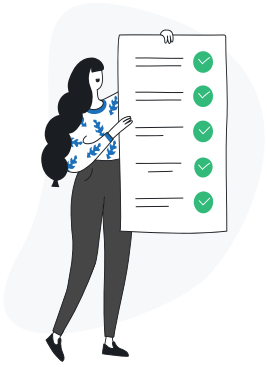
Chemicals and Reagents Used:
• Glucose (used as standard)
• 2.5 N Hydrochloric acid (HCl)
• Sodium carbonate (Na
• Concentrated sulfuric acid (96% H
• Concentrated phenol

Equipment Required:
• Test tubes,
• beakers,
• conical asks
• Measuring cylinders,
• funnel,
• micropipette
• Filter paper,
• mortar and pestle
• Hot air oven,
• water bath
• UV-visible spectrophotometer
• Magnetic stirrer
Preparation of Donut Samples:
i. Drying:
Donuts were rst dried using a hot air oven maintained at 50–60°C for 2 to 3 days to remove moisture.
ii. Grinding:
Once dried, the samples were nely powdered using a mortar and pestle.

thoroughly to obtain a uniform mixture.

Experimental Procedure:

i. Hydrolysis of Glucose Standard:
Weigh 100 mg of glucose into a test tube
Add 5 ml of 2.5 N HCl.
Mixture was heated in water bath
ii. Neutralization:
After hydrolysis, gradually add solid sodium carbonate until the bubbling (effervescence) stops. This indicates neutralization.
iii. Dilution:
Filter the solution by distill water. This forms your glucose standard solution.
iv. Preparation of Standards and Samples:
standard solution was made of 0.1- 0.5 ml
Add 0.1 ml of the sample extract into another test tube.
Bring the volume in each test tube up to 1 ml with distilled water.
v. Colour Development:
Add phenol
Carefully add 2.5 ml of concentrated sulfuric acid (H_2SO_4) to each, mix gently.
Let the mixture stand for 10 minutes, then shake gently.
Incubate the tubes in a water bath maintained at 25–30°C for 20 minutes.
(Note: Under acidic and heated conditions, carbohydrates like glucose are converted to furfural derivatives which then react with phenol to form a yellow-orange complex.)
vi. Measurement:
Measure the absorbance of each solution at 490 nm using a UV-visible spectrophotometer.
Use the standard readings to generate a calibration curve and estimate the carbohydrate content of the donut samples



No Plagiarism Found

SmallSEOTools

Plagiarism Scan Report By

Report Generated on: May

0%
Plagiarized

0%
Exact

0%
Partial

100%
Unique Content

Total Words: 15 Total Characters: 89 Plagiarized Sentences: 0 Unique Sentences: 1

Content Checked for Plagiarism

Protein Estimation Using BSA (Bovine Serum Albumin) Standard Curve

Required
BSA stock solution (0.3 mg/mL) Phosphate Buffered Saline (PBS)Reagents A- Folin Ciocalteu. B- BSA solution and Reagent C- Alkaline copper sulfate of Unknown protein sample (prepared mixing 500 uL unknown + 500 uL

Procedure:
i. Prepare a range of BSA dilutions in PBS to achieve standard concentrations between 30–150
ii. Set up the following
• Tube 1: Blank (only
• Tubes 2–6: BSA standards for
• Tube 7: Unknown
iii. Incubate all tubes at 50°C for 10 minutes, then allow to cool to room
iv. Reagent B is added 0.1ml it is mixed and
v. Then 3 mL is added in Reagent
vi. Re incubate at 50°C for 10 minutes, then let them cool for up to 30
vii. Record absorbance at 650 nm using a UV-VIS

No Plagiarism

SmallSEOTools

Plagiarism Scan Report By SmallSEOTools

Report Generated on: May 24,2025

0%
Plagiarized Content

0%
Exact Plagiarized

0%
Partial Plagiarized

100%
Unique Content

Total Words:147 Total Characters:1021 Plagiarized Sentences:0 Unique Sentences:7 (100%)

Content Checked for Plagiarism

Sugar Estimation via Fehling's Titration Method-

Apparatus:
- Burette
- Pipette
- Conical ask
- Volumetric ask
- Beaker
- Weighing bottle
- Funnel
- Wash bottle

Chemicals:
- Glucose
- $CuSO_4$ (Copper sulfate)
- NaOH (Sodium hydroxide)
- Sodium Potassium Tartrate (Rochelle Salt)
- Methylene Blue

Solution Preparation:
Fehling's A: Dissolve 17.32 g $CuSO_4$ in distilled water and make up to 50 ml

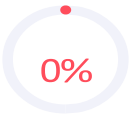
Fehling's B: solution is prepared by dissolving sodium potassium tartrate and sodium hydroxide in water.
Indicator: 1% Methylene blue in distilled water
.
Titration Setup:
- Mix equal volumes of Fehling's A and B (0.1 mL each).

Set up three test tubes:
Standard A: 500 µL glucose solution (no indicator)
B1 (Homemade): 500 µL of homemade donut extract
B2 (Monginis): 500 µL of Monginis donut extract

SmallSEOTools

Plagiarism Scan Report By SmallSEOTools

Report Generated on: May 24,2025



Plagiarized Content

0% Exact Plagiarized

0% Partial Plagiarized

Unique Content

Total Words:134 Total Characters:743 Plagiarized Sentences:0 Unique Sentences:8 (100%)

Content Checked for Plagiarism

Fat Extraction via Acid Hydrolysis-

Required Chemicals:

- 95% Ethanol
- Anhydrous Ethyl Ether
- Petroleum Ether
- 25.11% Hydrochloric Acid (HCl)
- Distilled Water

Steps:

i. Grind and sieve the dried sample (1 mm mesh).
ii. Add 1 mL of 95% ethanol to moisten.
iii. Introduce 5 mL of 25.11% HCl.
iv. The mixture is heated in the waterbath.
v. Cool to room temperature.
vi. Add 5 mL ethanol and mix.
vii. Ethyl ether is added and is shaken.
viii. Add and shake with Petroleum Ether
ix. Layers are seperated using pipette and it is ltered.
xi. Evaporate solvents on a low-temperature hot plate (~1 hour).
xii. Dry in an oven at 135°C for 10 minutes.
xiii. Cool and weigh the beaker. Fat content = nal weight – initial weight of the beaker.

No Plagiarism Found

SmallSEOTools

Plagiarism Scan Report By SmallSEOTools

Report Generated on: May 24,2025

Plagiarized Content

0% Exact Plagiarized

0% Partial Plagiarized

Unique Content

Total Words:174 Total Characters:1052 Plagiarized Sentences:0 Unique Sentences:9 (100%)

Content Checked for Plagiarism

Microbial Analysis-
A. Spread Plate Method (Quantitative Assessment)
Materials:
• Sterile Petri dishes
• Nutrient agar
• Pipette/inoculating loop
• Ethanol
• Marker
• Incubator
Steps:
i. Pour ~20 mL of sterile molten nutrient agar into plates and allow it to solidify.
ii. Label plates with sample name, dilution, and date.
iii. (If applicable) Prepare serial dilutions.
iv. To add 0.1 mL of a sample to an agar plate
v. Use a sterile spreader or glass rod to evenly distribute.
vi. Let the plates sit for 5–10 minutes.
vii. Incubation is done .
viii. Observe and count colony-forming units (CFUs)
B. Streak Plate Method (Pure Culture Isolation)

Steps:
i. The agar plate's bottom is labeled with the sample name, date, and any other relevant information.
ii. Loop is sterilize by heat and let it cool.
iii. Take culture and streak.
iv. Sterilize the loop again and allow it to cool before streaking into the second quadrant.
v. Repeat until four-quadrant streaking is complete.
vi. Incubate it for 24–48 hours.
vii. Examine for isolated colonies.

SmallSEOTools

Plagiarism Scan Report By SmallSEOTools

Report Generated on: May 24,2025

0%
Plagiarized Content

0%
Exact Plagiarized

0%
Partial Plagiarized

100%
Unique Content

Total Words:102 Total Characters:662 Plagiarized Sentences:0 Unique Sentences:1 (100%)

Content Checked for Plagiarism

Gram Staining (Bacterial Identi cation)-
Required Materials:
• Slides,
• Inoculating loop,
• Stains (crystal violet, iodine, safranin),
• 95% alcohol,
• Microscope

Procedure:
i. A thin smear of the bacterial culture is prepared on a clean microscope slide. This allows for better visualization and staining of individual cells.
ii. Air dry and heat- x the smear.
iii. Crystal violet is a primary stain that binds to the cell walls of both Gram-positive and Gram-negative bacteria.
iv. Add Gram's iodine (1 min), rinse.
v. Decolorize with alcohol (10–20 sec), rinse.
vi. Counterstain with safranin (1 min), rinse and blot dry.
vii. Examine under microscope.

No Plagiarism Found

SmallSEOTools

Plagiarism Scan Report By SmallSEOTools
Report Generated on: May 24.2025

0%
Plagiarized Content

0%
Exact Plagiarized

0%
Partial Plagiarized

100%
Unique Content

Total Words: 241 Total Characters: 1437 Plagiarized Sentences: 0 Unique Sentences: 12 (100%)

Content Checked for Plagiarism

Biochemical Test-
Catalase Test–
• Hydrogen peroxide was added
• Immediate bubble formation indicated a positive catalase reaction.

Indole Test-
• The bacterial culture was incubated for 48 hours.
• Reagent of Kovac is poured
• A ring appear which is pink in colour

Simmon Citrate Test:
• The pure colony was streaked into an agar slant.
• After 48 hours colour changes from green to blue which proves it as a positive citrate test.

Methyl Red Test (MR)
• The Methyl Red test is a biochemical method used to determine whether a microorganism performs mixed acid fermentation when utilizing glucose. In this process, some bacteria produce stable acidic end products, which lower the pH of the medium.
• Red colour gives positive results while yellow colour gives negative results

Voges-Proskauer Test (VP)
• After incubation in MR-VP broth, add:
• 0.6 mL of alpha-naphthol.
• 0.2 mL of KOH solution.
• It is mixed to observe the change of colour
Glucose Fermentation Test-
• Inoculate bacteria into glucose fermentation broth containing phenol red and a Durham tube.
• Incubate at 37°C for 24-48 hours.
• Check for:
• Colour change (acid production).
• Gas bubble formation (gas production).

Urease Test –
• Inoculate the test organism into a tube or slant containing urea broth or agar with phenol red indicator.

SmallSEOTools

Plagiarism Scan Report By SmallSEOTools

Report Generated on: May 25,2025

Plagiarized Content

0% Exact Plagiarized

0% Partial Plagiarized

Unique Content

Total Words:195 Total Characters:1212 Plagiarized Sentences:0 Unique Sentences:11(100%)

Content Checked for Plagiarism

Assessment of MIC for SAPP and Pectin in Donut Samples
Procedure
1. Using a sterile inoculation loop, bacterial colonies isolated previously on nutrient agar plates (e.g., E. coli and S. aureus) were transferred into LB broth.
2. Keep in a shaker at 150 rpm.
3. Incubation continued until the bacterial culture reached optical density (OD600) of ~0.6, indicating logarithmic phase growth.
4. This culture was used as the standardized inoculum for MIC testing.
Procedure for MIC Testing
1. Add the calculated volume V1 of each preservative stock to 5 mL of bacterial inoculum in sterile test vials.
2. Set up controls with inoculum but no preservatives.
3. Incubate and keep it for shaking
4. Measure OD600 every hour for 3 hours to monitor bacterial growth.
5. MIC is de ned as the lowest preservative concentration that visibly inhibits bacterial growth, indicated by stagnant or decreasing OD compared to control
Interpretation
- The MIC of SAPP for E. coli can be approximated at 100 µg/mL because bacterial growth is signi cantly inhibited at and above this concentration.
- The MIC of Pectin is slightly higher, approximately 200 µg/mL, due to comparatively less reduction in OD at lower concentrations

SmallSEOTools

Plagiarism Scan Report By SmallSEOTools

Report Generated on: May 25,2025

Plagiarized Content

0% Exact Plagiarized

0% Partial Plagiarized

Unique Content

Total Words: 290 Total Characters: 2156 Plagiarized Sentences: 0 Unique Sentences: 16 (100%)

Content Checked for Plagiarism

Results and Discussion-

The experiment compared microbial growth in homemade donuts and Monginis donuts over a period of time, both fresh and after storage. Microbial tests showed that the homemade donuts began developing visible signs of spoilage such as mold and foul odor within 3–5 days at room temperature. By the second week, there was signi cant bacterial and fungal growth, indicating high microbial activity due to the absence of preservatives.

In contrast, Monginis donuts exhibited much slower microbial development. Even after two weeks, the commercial donuts maintained a relatively stable appearance, odor, and texture. Microbiological tests con rmed fewer bacterial colonies and limited fungal presence, highlighting the effectiveness of the added preservatives—E450 (diphosphates) and E440 (pectin)—in delaying spoilage.

Preservative action was further con rmed by performing sugar, fat, protein, and carbohydrate tests on fresh and spoiled samples. While both donuts initially showed similar nutrient compositions, the homemade donuts showed signs of nutrient breakdown as microbial load increased, whereas Monginis samples remained more stable.

The results support the hypothesis that preservatives signi cantly improve shelf life and microbial resistance in processed food. However, the discussion must go beyond just stability. Although E450 and E440 are permitted food additives, regular consumption could in uence gut microbiota, especially in individuals with sensitive digestive systems. Some studies suggest that certain preservatives may interfere with bene cial gut bacteria, potentially leading to long-term health effects.

This trade-off between microbial stability and health implications is critical. While preservatives offer convenience and safety from spoilage, excessive reliance on them raises nutritional and microbiological concerns. Therefore, the food industry must consider using natural antimicrobial agents or reducing synthetic additive levels while maintaining food quality and safety. This study emphasizes the importance of informed consumer choices and the need for balanced food preservation methods.

No Plagiarism Found

SmallSEOTools

Plagiarism Scan Report By SmallSEOTools

Report Generated on: May 25,2025

Plagiarized Content

0% Exact Plagiarized

0% Partial Plagiarized

Unique Content

Total Words: 265 Total Characters: 1961 Plagiarized Sentences: 0 Unique Sentences: 11 (100%)

Content Checked for Plagiarism

Conclusion-

This study explored the microbial stability and preservative impact on homemade donuts compared to Monginis commercial donuts. Results clearly indicated that Monginis donuts, which contain food-grade preservatives such as E450 (diphosphates) and E440 (pectin), exhibited a signi cantly longer shelf life. These preservatives effectively suppressed microbial growth, even in samples stored for two weeks, thereby maintaining product safety and texture. In contrast, the homemade donuts—prepared without added preservatives—showed early signs of microbial spoilage such as mold growth, unpleasant odor, and changes in texture, especially after a few days of storage at room temperature.

Although preservatives help extend the shelf life of commercial baked goods and prevent rapid spoilage, their consistent consumption raises concerns about long-term health effects, including potential disruptions to the gut microbiota. While preservatives like E450 and E440 are permitted within safe limits, their impact on the gut environment, especially when consumed frequently, requires deeper investigation.

The ndings emphasize a trade-off between convenience and health: commercial donuts are more durable and appealing for mass production and distribution, but they rely heavily on chemical additives to maintain quality. Homemade donuts, though fresher and free from synthetic preservatives, are more vulnerable to spoilage and require immediate consumption or cold storage.

Ultimately, this comparison reveals the critical role preservatives play in food safety and shelf life while also drawing attention to the potential health implications of long-term consumption. Future research should focus on developing natural preservative alternatives that can offer similar antimicrobial bene ts without compromising human health. This would help balance consumer demand for both safety and nutrition, especially in baked goods that are widely consumed.

No Plagiarism Found

SmallSEOTools

Plagiarism Scan Report By SmallSEOTools

Report Generated on: May 25,2025

Plagiarized Content

0% Exact Plagiarized

0% Partial Plagiarized

Unique Content

Total Words: 823 Total Characters: 5492 Plagiarized Sentences: 0 Unique Sentences: 31 / 100%

Content Checked for Plagiarism

Appendix-

Appendix A: Materials Used
1. Samples
• Homemade donuts (prepared without preservatives)
• Monginis donuts (store-bought, containing E450 and E440)

2. Chemicals & Reagents
• Fehling's Titration Method(for sugar test)
• Biuret reagent (for protein test)
• Acid Hydrolysis (for fat test)
• Phenol sulphuric acid (for carbohydrate)
• Distilled water
• Agar media (Nutrient agar, LB broth)
• Food preservatives: E450 (Sodium Acid Pyrophosphate), E440 (Pectin)

3. Equipment
• Test tubes and racks
• Microscope
• Incubator
• Petri dishes
• Sterile swabs
• Measuring cylinders
• Bunsen burner
• pH meter
• Refrigerator
Appendix B: Method Summary
1. Preparation of Samples
Freshly prepared homemade donuts stored at room temperature.
Monginis donuts purchased on the same day, stored under the same conditions.

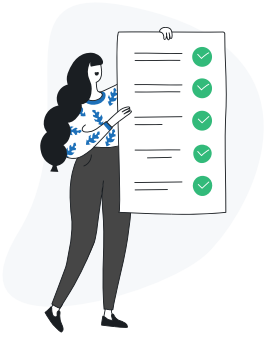
2. Biochemical Tests
Sugar, fat, protein, and carbohydrate tests were conducted on both fresh and stored donut samples to assess nutrient breakdown.

3. Microbial Testing

Microbes was streak on agar plate and was incubated for 72 hours
Colony growth observed and recorded on Day 3, Day 7, and Day 14.

4. Preservative Testing
Effects of E450 and E440 were analyzed by observing microbial growth inhibition and nutrient preservation.

Appendix C: Observations (Sample Format)
Sample Type Day 1 Day 7 Day 14
Homemade Donut No growth Fungal spots Heavy microbial load
Monginis Donut No growth Minimal spots Mild microbial activity



No Plagiarism Found